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Morpho-Histology of Gut and Microbiological Study on Gut Content of Earthworms of Rajshahi University Campus

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Morpho-histology of gut and microbiological study on gut content of earthworms of Rajshahi University Campus

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Dedicated
To My
Beloved
Parents

DECLARATION

I Md. Fazlul Haque declare that the thesis entitled "Morpho-histology of gut and microbiological study on gut content of earthworms of Rajshahi University Campus" is the bonafide record of the original research work carried out by me under the supervision of Prof. Dr. Ananda Kumar Saha and that it has not been submitted earlier elsewhere for the award of any degree, diploma or fellowship.

29/6/09

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CERTIFICATE

This is to certify that the thesis entitled "Morpho-histology of gut and microbiological study on gut content of earthworms of Rajshahi University Campus" is the bonafide record of the original research work carried out by Md. Fazlul Haque under my supervision and that it has not formed the basis for the award of any degree, diploma or fellowship.

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The Author

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Abstract

Earthworm plays an important role on economy and society of Bangladesh which is based on agriculture. A fertile and productive soil is the fundamental resource for sustainable Agriculture. Earthworm can play a variety of important roles in fertility and productivity of soil by providing shelter to the beneficial microbes in their gut and by activating soil microbial activity through the excretion and casting. Many microorganisms in the soil remain in the dormant stage, awaiting suitable habitat for the vigorous multiplication. Earthworm gut is suitable habitat for many microorganisms, but not for all. Many microorganisms may be digested in gut. Therefore, present investigation was done to find out the morpho-histological and microbiological variation in gut of different earthworm species. Then, investigation was done to ascertain the correlation between morpho-histological variation and microbial variation of gut of different earthworm species. Rajshahi University campus was the study area of the present research work. Three types of habitates i.e. shady land, crop land and drainage of residential hall were selected for study from September, 2004 to August, 2005. The fortnightly sampling dates were fixed on 14th day for first sampling and 28th day for 2nd sampling each month. Quadrate sampling and Hand sorting methods were use to collect earthworm. Collected earthworms were identified with an identification key. Data on weather were collected from Regional Weather Office of Rajshahi. For morphological study, earthworms were dissected and cleaned to separate the gut. Permanent slides of gizzard, stomach and intestine of the gut were prepared and then observed by advanced biological microscope for histological study. Bacterial load of gut of earthworm was studied by serial dilution method and spread plate technique on nutrient agar plate. Finally, pure culture of bacteria isolated from the different parts of gut of earthworms were tested for Gram character, morphology, motility, catalase

and oxidase reactions, citrate utilization and coagulase test. The isolates were then identified with Bergey's manual of systematic bacteriology. Six species of earthworm i.e *Metaphire posthuma*, *Eutyphoeus orientalis, Eutyphoeus nicholsoni, Eutyphoeus incommodus, Lampito mauritii* and *Drawida limella* were identified from three studied habitats. Earthworm diversity was present in the studied habitats. The number of earthworms also varied with month. In most cases, higher number of earthworm was recorded on July, August and September. On the other hand, lower number of earthworm was recorded from November to April. Morphological study of gut of earthworm revealed that number, length and position of different parts of gut of all studied earthworm species were not same. Result of histological study showed that same layers of muscle and cuticle were presence in gut of all studied species of earthworm, but some species ways variation were found in the width of the different layers of the gut. Result of bacterial load study showed that bacterial load of gut vary with part of the gut, species of earthworm and season or month of the year. But species ways variation of bacterial load was not statistically significant. Analysis of correlation indicates that bacterial load of one part gut is correlated with that of other parts of gut and also with the components of weather. The highest bacterial load of gizzard was recorded in *D. limella* on September while the lowest load was recorded in *E. nicholsoni* on January. Bacterial load of stomach of *E. orientalis* was higher than that of the other species from January to May, but that of *E. nicholsoni* was higher than that of the other species from June to December. Bacterial load of stomach of *D. limella* was lower than that of the other species from December to July. But, miscellaneous result was found on lower bacterial load from August to November. The highest bacterial load of intestine was recorded in *E. nicholsoni* on September while the lowest load was recorded in *E. incommodus* on December. Five genera of bacteria were identified from six species of earthworm. These were *Bacillus, Pseudomonas,*

Klebsiella, Streptococcus and *Acinetobacter.* No remarkable variation of bacterial fauna was found in studied species of earthworm. In other ward, these five types of bacteria were found in all studied species of earthworm. It can be concluded that there are morpho-histological variation in gut of different earthworm species. But, morphological variation was more prominent than the histological variation of gut of earthworm. One other hand, insignificant species ways variation of bacterial load and uniform types of gut bacteria reveal that there is no species specific bacterial profile for gut of different earthworm. Therefore, form the data of this study it is not possible to establish a method for identification of an earthworm species depending on their bacterial profile of gut.

Introduction

Bangladesh economy and society is based on agriculture, providing large part of the gross domestic product (GDP). A fertile and productive soil is the fundamental resource for sustainable farming. Earthworm can play a variety of important roles in fertility and productivity of soil. Earthworms are natural invertebrates of agro ecosystem belonging to the phylum Annelida, class oligochaeta and family lumbricidae. They lack suckers and parapodia but possess setae on all segments except the peristomium and pygomers. They are dominant in the temperate and tropical soils. Earthworms are one of the most important organisms among soil invertebrates owing to their beneficial effects on soil environment such as modification of soil physical properties and impact on decomposition of soil organic matter (Lee, 1992; Wolters, 2000). Its significance was recognized by Darwin (1890) as early as 1837 when He began his observations on the abundance of earthworms and their effects on soil. In 1878 the Danish soil scientist P. E. Muller (Cruickshank, 1972) identified earthworm activity as a crucial element involved in the genesis of forest soils.

The most effective use of earthworms in organic waste management requires a detailed understanding of biology of all potentially useful species (Edward, 1998). Population dynamics and productivity in earthworms can not be fully understood unless the life cycle of each earthworm is known. There are studies on life cycle and reproductive strategies of earthworms on temperate species (Lavelle, 1979), Indian species (Julka, 2001) and tropical species (Dash & Senapati, 1980). Knowledge of reproductive strategies of earthworms comes predominately from studies on temperate species (Jimenez *et al.*, 1999). Studies on the life cycles i.e. cocoons production, morphology, hatching pattern and fecundity of seven tropical earthworm species have been done by Battacharjee and Chaudhari (2002) for effective vermiculture.

Earthworms are hermaphrodites, both male and female reproductive organs are present in every single earthworm but self-fertilization does not generally occur. At the time of laying eggs, the sexually mature worms have a distinctive epidermal ring shaped area called, the clitellum, which has gland cells that secrete material to form a viscid, girdle like structure known as cocoon. Cocoons are small, with their size varying according to species. The colour of the cocoon changes gradually as it develops from the freshly laid stage to the hatching stage. Though the number of fertilized ova in each cocoon ranges from one to twenty for lumbricid worms (Stephenson, 1930), often only one or two survive and hatch (Edwards & Lofty, 1972). Cocoon production starts at the age of 6 weeks and continues till the end of 6 months. Under favourable conditions one pair of earthworms can produce 100 cocoons in 6 weeks to 6 months (Ismail, 1997). The incubation period of a cocoon is roughly about 3-5 weeks, in temperate worms it ranges between 3-30 weeks and in tropical worms within 1-8 weeks. Earthworms also have the power to regenerate segments, which are lost.

Quality of organic waste is one of the factors determining the onset and rate of reproduction (Dominguez *et al.*, 2000). The quantity of food taken by a worm varies from 100 to 300 mg/g body weight/ day (Edwards & Lofty, 1972). Earthworms derive their nutrition from organic materials, living micro organisms and by decomposing animals. Surface living earthworms feed on food material selectively while deep soil living worms ingest soil as such. The type and amount of material available influence the size of earthworms, population, species diversity, growth rate and cocoons production.

Earthworms are generally classified as saprophages but based on their feeding habits they are classified into detrivores and geophages (Lee, 1985). Detrivores feed at or near the soil surface on plant litter or dead roots and other plant debris or on mammalian dung. These worms are called humus formers and comprise the epigeic and anecic forms. *Perionyx excavatus, Eisenia fetida, Eudrilus euginae, Lampito mauritii, Polypheretima elongata, Octochaetona serrata* and *Octochaetona curensis* are few examples of

detrivorous earthworms (Ismail, 1997). Geophagous worms, feeding beneath the surface, ingest large quantities of organically rich soil and comprise the endogeic earthworms; *Metaphire posthuma* and *Octochaetona thurstoni* are two common examples of geophages.

Epigeics are surface dwellers and feed on organic matter on soil surface. Endogeic earthworms spend most of their time in the minerals layer of soil and burrow predominantly. Anecic earthworms like *Lumbricus terrestris* predominantly make vertical burrows. Of these three ecological varieties of earthworms, the epigeics and anecics have been harnessed for use in the vermicomposting process.

Earthworms have been shown to play an important role in litter decomposition and maintenance of soil fertility in the surface layers of the soil (Syers & Springett, 1984). Most of the studies available on earthworm ecology are from temperate countries, where species largely belong to the family Lumbricidae (Edwards, 1983). In contrast, the earthworm species of tropical soils belong to a wider variety of families, such as the Almidae, Kynolidae, Megascolecidae, Endritidae and Ocnerodrilidae, about which little is known except for some preliminary reports on natural ecosystems such as grasslands (Dash & Patra, 1977) and woodlands (Krishnamoorthy, 1985).

Earthworm ingests a variety of organic materials from soils (Hughes *et al.* 1994), that differ in quantity and chemical and physical palatability over a heterogeneous landscape. These include leaf litter, living and dead roots, microbial biomass, animal manure, leaf leachates, and root exudates (Lee, 1985., Scheu, 1987., Lavelle, 1988., James, 1992). In tropical forest , canopy leaf litter has been suggested to be the major resource for decomposer communities (including earthworms) due to high carbohydrate content (Satchell & Lowe, 1967., Martin & Lavelle, 1992). Some studies have described the relationship between earthworm abundance and food resources by manipulating animal dung in agro-ecosystem (James, 1992., Hughes *et al.,* 1994). Very few studies have dealt with the dynamics of

earthworm communities as influenced by plant species and their litter input in tropical ecosystems. A recent study has shown that the density and fresh weight of earthworms were twice as high in a *Dacryodes* community occurring along ridges than in a *Heliconia* community occupying valleys within a tropical wet forest in Puerto Rico (Gonzales, 1996).

Much of the information available on the ecology of earthworms (Annelida: Oligochaeta) in forest ecosystems is from the temperate regions of the world (Edwards, 1983). Earthworms play an important role in determining the nutrient cycling pattern through their role in litter decomposition and soil turnover (Syers & Springett, 1984). The information on the ecology of earthworms from natural ecosystems of the tropics is limited. The species here largely belong to families such as the Almidae, Kynolidae, Megascolecidae, Endritidae and Ocnerodrilidae (Dash & Patra, 1977., Krishnamoorthy, 1985) while the temperate species largely belong to the family Lumbricidae (Satchell, 1983). The tropical species are largely surface soil dwellers (Bouche, 1977) unlike the deep burrowing Lumbricids (Bouche, 1977), as shown through earlier studies (Bhadauria & Ramakrishnan, 1989).

Soils are perhaps the most complex microbial habitat on earth (Tate, 1995) and estimates on the microbial loads of soil range from 104 to 106 distinct prokaryotic genomes (i.e., species) per gram dry weight soil (Curtis *et al.,* 2002., Gans *et al.,* 2005). Assuming a gut volume of 450 cubic mm (100 mm × 1.44mm2 × π) per worm and worm densities of up to 2000 individuals per square meter (Edwards, 2004., Edwards & Bohlen, 1996.), each square meter of soil can contain nearly one liter of earthworm gut. Thus, the potential impact that gut passage has on soil microorganisms and associated processes is of general importance to soil microbiology and the geoecology of terrestrial habitats.

The gut of earthworms consists of a pharynx, oesophagus and gizzard followed by an anterior intestine that secretes enzymes and a posterior intestine that absorbs nutrients. During progress through digestive system

there is a dramatic increase in number of micro organisms of upto 1000 times. There is experimental evidence that micro organisms provide food for earthworms. Bacteria are of minor importance in the diet, algae are of moderate importance and protozoa and fungi are major source of nutrients. Worms, produced under sterile conditions could live on individual cultures of certain bacteria, fungi and protozoa but grew best on various mixtures of micro organisms. In order to study the modification of microflora during vermicomposting of rabbit manure the numbers of micro-organisms of various groups were determined during and after the process and compared with those resulting from a parallel spontaneous maturation process of the same material. Actual vermicomposting brought out less change in microbial counts analogous to during a prolonged spontaneous maturation of rabbit manure (Allievi *et al.*, 1986). However, specific nutritional interactions were observed between *E. fetida* and micro-organisms. The earthwoms were found to be feeding directly upon the cells of certain micro-organisms. Other species were found to be toxic to *E. fetida*. The seeding of vermiculture beds with the bacterium *Acinetobacter calcoaceticus* stimulated earthworm growth and consumption of the substrate, while no difference was observed for *Acetobacter diazotrophicus* inoculation over the worm reproductively (Hand, 1988).

Earthworm influence soil structure by providing shelter to the beneficial microbes in their gut and by activating soil microbial activity through the excretion and casting (Dash *et al.,* 1986). Earthworms voraciously feed on organic wastes and while utilizing only a small portion for their body synthesis they excrete a large part of these consumed waste materials in a half digested form. Since the intestines of earthworms harbour wide ranges of microorganisms, enzymes, hormones, etc., these half digested material decompose rapidly and is transformed into a form of vermicompost within a short time (Edwards & Lofty, 1972; Kale & Bano, 1986).

Earthworms are also 'ecosystem engineers' as they actively redesign the physical structure of the soil environment by their activities of ingesting litter

and soil particles, depositing casts on the soil surface and translocating soil particles while their gut, within which many changes take place, have been described as 'natural bioreactors'. The activities of earthworms in soils have been shown to have profound impart on the soil ecosystem functioning as well as on the types and numbers of micro-flora and micro-fauna (Pederson & Hendriksen, 1993). Major part of their beneficial effects on soil properties is attributed to their feeding activities and interactions with soil microorganisms (Edwards & Fletcher, 1988) because their consumption pattern involves the breakdown and incorporation of large amounts of mineral soil and organic matter (Piearce, 1978) which contains a variety of microorganisms. During passage of microorganisms along with organic residues through the earthworm's intestinal tract, their population may increase. Earthworm casts have been reported to be much more microbiologically active and richer in micro-flora than their surrounding un-ingested soils (Scheu, 1987., Daniel & Anderson, 1992). It has also been reported that while earthworms use organic matter as their nutrient source, the microorganisms ingested along with these nutrient sources actually elaborate the enzymes that make the nutrients available for the worm's use (Edward & Lofty, 1972., Lee, 1985). It is likely that the ingested microbial populations play a key role in earthworm nutrition by helping in the breakdown of organic matter, particularly the components that the earthworms cannot utilize in their natural state (Hornor & Mitchell, 1981).

Several recent studies have shown that earthworms can mediate bacterial transport into different environments (Daane *et al.*, 1997). Stephens *et al.*,(1993) reported that the transport of beneficial bacteria by earthworms were important to the biological control. Due to these characteristics, earthworms act as vectors for the dispersal of soil microorganisms (Madsen & Alexander, 1982) through lateral and vertical zones, and as bioreactors for certain kinds of microorganisms (Parle, 1963). However, several differences exist between the gut condition of earthworm and soil environment (Egert *et al.*, 2004). To survive throughout gut passage, bacteria must adapt to the

anaerobic and physicochemical gut conditions (Horn *et al.,* 2003) the lysis of microbes by digestive enzymes secreted by the earthworm (Edwards & Fletcher, 1988) and the inhibition of bacteria by inhibitory substances secreted by other bacteria (Brown, 1995). Therefore, to facilitate the use of earthworms as vectors for the dispersal of beneficial bacteria, the facultative intestinal microorganisms in earthworms must be characterized (Kim *et al.,* 2004).

Many microorganisms in the soil remain in the dormant stage with low metabolic activity, awaiting suitable habitat for the vigorous multiplication of selected group of microorganism, which are stimulated to decompose ingested organic matter (Horn *et al.,* 2003). Earthworm guts are suitable habitat for microorganism. The gut of earthworms consists of a pharynx, oesophagus and gizzard followed by an anterior intestine that secretes enzymes and a posterior intestine that absorbs nutrients. During progress through digestive system there is a dramatic increase in number of micro organisms of upto 1000 times (Allievi, 1986). Therefore, investigation should be done to find out the morpho-histological and microbiological variation in gut of different earthworm species. Investigation also should be done to find out that the microbial variation of gut is either species specific or other factors dependent. If microbial variation of gut is species specific then an attempt will be taken to establish a new method of identification of earthworm on the basis of their gut microbial profile.

The Objectives of Research Work

Based on these facts the present study aims at the following objectives:

- To study the abundance of earthworms at Rajshahi University campus
- To study the morpho-histology of gut of earthworm species
- To study the bacterial load of gut of earthworm species
- To study the bacterial variation in gut of different species of earthworms available at Rajshahi University campus

Review of Literature

There are about 3000 species of earthworms distributed all over the world and about 384 species are reported from India (Julka, 1986). In Bangladesh the major taxonomic work on earthworm, so far done are of Stephenson (1923), who reported 18 species, mainly from Rajshahi, Comilla, Chittagong Hill Tracts, Rangamati and Sundarbans areas. Julka & Senapati (1987) and Julka (1988) mentioned the presence of 12 species in India. Reynoldes *et al.* (1995) reported 20 species of earthworm from Bangladesh. Jahan *et al.* (1999) reported 6 species of earthworms from Bogra district. Mannan *et al.* (1994) worked on biology of the earthworm, *Metaphire posthuma* with special emphasis on its population density. A Survey bionomics of earthworm fauna of Rajshahi University campus was carried out by Abdullah (1992) (Mannan *et al.*, 1994; Ali *et al.*, 1995) and found 8 species.

Earthworms are found in almost all types of soil in forests, grasslands, arable lands and gardens with sufficient moisture and food. They are omnivorous and mostly derive nutrition from dead organic matters (Jahan *et al.*, 1999). Population density of earthworm is dependent on different environmental factors. Quality of organic waste is one of the factors determining the onset and rate of reproduction (Dominguez *et al.*, 2000). Earthworm population contributes 80% of the total biomass of the soil ecosystem (Mukherjee and Singh, 1986).

There are about 1500 species of earthworm, ranging in size from less than 1 mm long to the gigantic Australian worm which attains a length of 10 to 11 feet. The common earthworm, *Lumbricus terrestris,* rearely exceeds 9 or 10 inches in length. It is abundant in moist rich soil (Mukherjee an Singh, 1986). Although earthworm species differ in size and behaviour, the general activities of all species are similar (Edwards & Lofty, 1972). They move through the soil, displacing and ingesting mineral and organic matter. Earthworms especially favour dung, succulent herbage (grass), and tree

leaves. The leaves of ash, hickory, tulip tree, dogwood, and basswood are among the most favoured: foliage of oaks and conifers are least favored (Satchell 1967., Satchell & Lowe 1967., Gilbert & Bocock, 1960., Barley, 1959). Soil containing considerable organic matter or at least a layer of humus on the surface, maintained the largest earthworm fauna but other factor are important to the distribution of terrestrial species (Barnes, 1974). During the dry seasons or winter, earthworms migrate to deeper levels of the soil, down to loft in the case of certain Indian species. After moving to deeper levels, during dry period an earthworm often undergoes a period of quiescence losing as much as 70 percent of its water (Barnes, 1974).

The number of earthworms in an agricultural field is influenced by the intensity and number of soil disturbance events like tillage and traffic, the abundance and quality of food sources, the chemical environment of the soil, and the soil microclimate. Important factors of the soil environment include organic matter (food sources), soil type, depth to a restrictive layer, moisture holding capacity and internal drainage, rainfall and temperature, predation and parasitism, soil pH (Edwards & Lofty, 1977).

As the number and intensity of tillage operations increase, so does the physical destruction of burrows, cocoons, and the earthworm bodies themselves. Less intensive tillage systems that leave residues on the surface throughout the year improve the environment for earthworms. Decreased tillage disturbances particularly benefit night crawlers (*L. terrestris*), which move in the same burrow between deeper soil layers and the soil surface in search of food. Endogeic (shallow dwelling) earthworms will tolerate annual tillage because they continually form new burrows and acquire a greater proportion of their food from the soil rather than surface litter. No-till and other methods of conservation tillage such as chisel plowing and ridge tillage can increase populations of both types of earthworms (Edwards & Bohlen, 1996).

Although a single tillage event will not drastically reduce earthworm populations, repeated tillage over time will cause a decline in earthworm

populations (Edwards & Bohlen, 1996., Edwards *et al.,* 1995., Edwards & Lofty, 1977., Coleman & Crossley, 1996).

Nearly all organic fertilizers benefit earthworms. The addition of animal manure, sewage wastes, and spent malt from breweries, paper pulp, or potato processing waste all showed a positive effect on earthworm numbers (Edwards *et al.,* 1995). Additions of organic material can double or triple earthworm numbers in a single year. The ammonia and salt content of some liquid manure can have an adverse effect on earthworms, but populations usually recover quickly and henceforth increase (Edwards & Bohlen, 1996).

Normally, the use of inorganic fertilizers also has a positive impact on earthworm numbers. This is probably an indirect effect of the increased crop biomass production and consequent increases in organic residues (Edwards & Bohlen, 1996., Edwards *et al.,* 1995). Hendrix *et al.* (1992) reported that earthworm numbers in meadows receiving inorganic fertilizer averaged nearly twice the earthworms in unfertilized meadows on the Georgia piedmont.

Ammonia and ammonia-based fertilizers can adversely affect earthworms. Annual use of ammonium sulfate, anhydrous ammonia, and sulfur-coated urea has been shown to decrease earthworm populations (Edwards *et al.,* 1995). Research at Park Grass (Rothamsted) since 1856 showed that after extremely long exposure to several levels of ammonium sulfate (0, 48, 97, and 145 kg/ha), the populations of earthworms were inversely proportional to the dose of nitrogen applied (Edwards & Lofty, 1977). This is probably due to the effect these fertilizers have on lowering 6 soil pH. Direct exposure to anhydrous ammonia during application will kill up to 10% of the population. However, farmers report increased numbers in the long run due to higher yields and more food for earthworms to feed upon (Ernst, 1995). Still, some farmers have switched from anhydrous ammonia to 28% nitrogen to avoid killing earthworms during nitrogen application. Others have converted to using manures in order to protect and increase earthworms (Ernst, 1995). Lime seems to benefit earthworm populations in otherwise acid soils because most

species of earthworms favour neutral pH levels and require calcium for growth. Lime may indirectly benefit earthworms by increasing plant growth and therefore plant residues. A study in New Zealand showed a 50% increase in surface feeding earthworm species by adding one ton of lime per acre (Edwards *et al.*, 1995).

In general, most herbicides are harmless to earthworms. The triazine class of herbicides has a moderate impact on earthworm numbers. Herbicides used prior to World War II, including lead arsenate and copper sulfate, are moderately toxic to earthworms. The main threat of toxicity to earthworms is from long-term buildup of these compounds in the soil (Edwards & Bohlen, 1996). The majority of the carbamate class of insecticides are toxic to earthworms. The toxic effects of carbofuran (Furadan) have been studied extensively. Other insecticides in the carbamate class that have proved highly toxic to earthworms are aldicarb (Temik), aminocarb, bufencarb, carbaryl (Sevin), methiocarb (Measural), methomyl (Lannate), oxamyl (Vydate), promecarb, propoxur (Baygon), and thiofanox. Generally, insecticides in the organophosphate class are less toxic to earthworms. However, organophosphate insecticides that are extremely or highly toxic are phorate (Thimet), chloropyrifos (Dursban, Equity, Tenure, etc.), ethoprophos (Mocap), ethyl-parathion, and isazophos. Aromatic organochlorine insecticides (used predominantly in the 1950.s-1970.s) are generally not very toxic. Exceptions are chlordane, endrin, heptachlor, and izobenzan. Carbamate fungicides (carbendazim and benomyl) have shown toxic effects to earthworms. Other broad-spectrum fumigants (fungicides and nematicides) are very toxic to earthworms (Ernst, 1995., Edwards & Bohlen, 1996).

Irrigated soil can support high levels of earthworm activity where moisture levels would otherwise be too dry. Irrigation also increases crop production, resulting in more food and increased earthworm populations. Irrigation waters that carry earthworms and their cocoons may act as a source of inoculum for certain species (Edwards *et al.*, 1995).

Earthworms feed selectively on material rich in organic matter, such as organic polymers (or breakdown products thereof) derived from plants, protozoa, fungi, and bacteria (Brown & Doube, 2004., Edwards & Fletcher, 1988). Ingested protozoan are required for maturation of certain earthworm species and are digested in the crop, gizzard, and foregut (Bonkowski & Schaefer, 1997., Miles, 1963., Piearce & Philips, 1980). Ingested fungal hyphae are greatly reduced in length during gut passage, whereas ingested fungal spores may remain intact (Brown & Doube, 2004., Schonholzer *et al.,* 1999., Wolter & Scheu, 1999). Digestion of large ingested bacteria may also occur during gut passage (Brown & Doube, 2004., Clegg *et al.,* 1995., Schonholzer *et al.,* 2002), although total and culture-dependent bacterial counts tend to increase (Fischer *et al.*, 1994., Parle, 1963., Pedersen & Hendriksen, 1993., Schonholzer, 2002., Wolter & Scheu, 1999). Thus, there is evidence that ingested microorganisms with high cell volumes are preferentially disrupted in the gizzard. The alimentary canals of *L. terrestris* and *E. foetida* are perhaps the most thoroughly described earthworm digestive systems (Breidenbach, 2002., Brown & Doube, 2004., Edwards & Bohlen, 1996., Edwards & Fletcher, 1988., Kukenthal & Renner, 1982., Laverack, 1963., Tillinghast *et al.,* 2001., van Gansen, 1963). Ingested material, which is usually a mixture of organic material and soil, enters the alimentary canal via the mouth, is transferred sequentially to the esophagus, crop, gizzard, intestine, and finally leaves the worm via the anus. Salivary glands in the pharynx modify ingested soil/litter with amylase- and protease-containing mucus that aids in the movement of coarse, dry material through the alimentary canal. Calciferous glands in the esophagus secrete mucus that contains calcium carbonate, enabling the worm to expel excess calcium and carbonate and to regulate the pH of gut and coelom fluids. A chitinous membrane (called a peritrophic membrane) (Arthur, 1963., Breidenbach, 2002) that lines the alimentary canal from the crop to the end of the midgut region has both protective and digestive functions. This membrane contains digestive enzymes that are released into the gut lumen when abraded by

ingested material as it passes through the alimentary canal. The gizzard is a muscular, cuticula-lined grinder that triturates ingested material. Digestive enzymes (e.g., lipases, chitinases, and cellulases) are secreted into the intestine by both the worm and ingested microorganisms. The intestinal mucus that is secreted in large quantities into the foregut contains water-soluble organic carbon that can be readily degraded by microbes (Lavelle *et al.,* 1995., Martin *et al.,* 1987., Trigo *et al.,* 1999). Digested microbial biomass yields forms of organic carbon that can be utilized by the earthworm. For example, long-chain fatty acids likely derived from the membranous lipids of digested bacteria can be assimilated into earthworm tissues (Sampedro *et al.,* 2006). Earthworms vary in their capacities to assimilate carbon and energy from ingested materials, and assimilation efficiencies range from 1% to 60% (Edwards & Bohlen, 1996). The earthworm gut has been described as a "mutualistic digestive system" (Barois & Lavelle, 1986., Brown & Doube, 2004.) in which the exoenzymes produced by ingested microorganisms enhance the degradation of complex organic matter during their passage through the gut and thus enhance the capacity of the worm to assimilate nutrients. In contrast to aerated soils that are subject to drying and fluxes of O_2, the earthworm gut is relatively moist and free of detectable O_2 . The high moisture content of the anterior parts of the digestive tract decreases toward the anus (Barois & Lavelle, 1986., Horn, 2003). The amount of water-soluble organic matter in the foregut and hindgut are approximately 30% and 4%, respectively, of the dry weight of gut contents; in contrast, the water-soluble organic matter of casts and soil are approximately 0.3% and 0.0%, respectively, of the dry weight of material (Trigo & Lavelle, 1993., Trigo & Lavelle, 1995). Thus, ingested microorganisms initially encounter an anoxic niche that is moist and rich in water-soluble organic matter that is derived in part from the breakdown of intestinal mucus and ingested biomass. The organic carbon available in the gut includes high-quality electron donors. For example, the aqueous phase of gut contents can contain more than 100mM glucose that is likely derived from the hydrolysis and degradation of the

mucus secreted into the alimentary canal. Large amounts of amino sugars and maltose can also occur in gut contents. Thus, the *in situ* conditions of the gut are ideal for anaerobic metabolism of ingested soil microorganisms. The occurrence of a large number of volatile fatty acids (e.g., formate, acetate, succinate, and lactate) in gut contents corroborates the likelihood that fermentative processes are ongoing in the gut. The concentrations of nitrate and nitrite are lower and higher, respectively, in the earthworm gut than in soil, suggesting that nitrate is subject to reductive processes in the gut. Ammonium and amino acids are abundant in the gut and would theoretically enhance the anabolism of ingested soil microorganisms. Indeed, essential amino acids differ qualitatively between the gut and soil, a finding that is consistent with the production of proteins by microbes during gut passage and/or the occurrence of earthworm-derived proteins in the gut (Pokarzhevskii, 1997). Despite the high concentrations of organic molecules and the metabolic dynamics of the earthworm gut, the pH of the gut is relatively neutral and less variable than that of soil, indicating that pH homeostasis occurs in the gut. Total carbon and total nitrogen of gut content are higher than those of soil, and the C/N ratio of gut content is lower than that of soil. The collective properties of the earthworm gut are consistent with the earthworm alimentary canal functioning as a mutualistic digestion system. As outlined in the preceding section, the anoxic conditions and availability of highquality organic carbon in the earthworm gut theoretically favor microorganisms capable of anaerobic growth. Although relatively few studies have systematically (i.e., simultaneously) quantified the microbiota of earthworm gut contents and preingested aerated soil (i.e., the soil inhabited by earthworms), in which the primary goal is to resolve quantitative differences between aerobic, anaerobic, and facultative microbes in these two contrasting materials, the information available indicates that cultured numbers of microbes capable of growth under anoxic conditions are higher in the gut than in the aerated soil from which earthworms are obtained (Karsten & Drake, 1997). Given the organic rich conditions in the anoxic gut, one might

project that significant qualitative differences would exist between the fermentative microbes cultured from gut contents and preingested soil. However, as illustrated by the product profiles of most-probable-number analyses, the major difference between fermentative microbes cultured from gut contents and preingested soil is quantitative, not qualitative. Kinetic patterns of fermentative processes indicate that the fermentative microbes in the earthworm gut are poised at a more active state than are those of soil (Drake *et al.,* 2006., Karsten & Drake, 1995). Exoenzymes of fermenters might augment the digestion of complex organic matter during gut passage (Bergey *et al.,* 1990). Although the *in situ* conditions of the gut would seem favourable for acetogens (Drake *et al.,* 2006) and methanogens (Whitman *et al.,* 2006), these two groups of obligate/strict anaerobes appear to not be enhanced or metabolically significant in the earthworm gut (Hornor & Mitchell, 1981., Karsten & Drake, 1995). Thus, in terms of general function, the types of anaerobes found in the gut appear to be qualitatively representative of the types of anaerobes found in soil. Despite the deficiency of molecular oxygen (O_2) in the gut, the viable counts of microbes capable of aerobic growth, including nitrifying bacteria, are also higher in the gut than in preingested soil (Karsten & Drake, 1997). Such findings support the conclusion that the number of cultured ingested aerobes increases during passage. However, certain observations do not support this conclusion. For example, the viable counts of aerobes in the foregut of *A. caliginosa* can be higher than those in the hindgut. Independent of such inconsistencies, the relative abundances (i.e., gut-derived versus soil-derived abundances) of cultured anaerobes and denitrifying bacteria are greater than those of cultured general aerobes. These findings are consistent with the contrasting *in situ* conditions of the anoxic earthworm gut and aerated preingested soil. On the basis of these collective observations, it can be argued that there is a general stimulation of ingested microorganisms in the earthworm. An alternative explanation for the quantitative differences in cultured microbes between gut content and soil is the occurrence of a high number of endemic gut microorganisms. Although

endemic gut species can be expected, for example gut wall-attached microbes (Jolly *et al.,* 1993., Mendez *et al.,* 2003), evidence to date for a quantitatively significant gut-specific microbiota is scant. Indeed, 16S rRNA genes retrieved from the earthworm gut are indicative of the microbial biome of preingested material (Egert *et al.,* 2004., Furlong *et al.,* 2002., Singleton *et al.,* 2003), and the strongest evidence to date for an earthworm-specific microbial symbiont is the occurrence of *Acidovorax* species in earthworm nephridia (Davidson & Stahl, 2006., Pandazis, 1931., Schramm *et al.,* 2003). In marked contrast to the greater cultured abundances of microbes in the earthworm gut than in preingested soil, culture-independent methods (e.g., staining with 4-,6-diamido- 2-phenolindole) paradoxically indicate that the microbial abundances in the gut and soil are only marginally different (Karsten & Drake, 1997). This paradox is addressed below. Culture independent methods indicate that the total number of microbial cells in the earthworm gut of *Lumbricus* spp. approximate 1 × 1010 microbial cells per gram dry weight of gut contents and that culture-dependent methods with anoxic solidified medium (i.e., anoxic roll tubes) resolved viable counts of 2 × 109 microbial cells per gram dry weight of gut contents (Karsten & Drake, 1997). Enumeration of viable counts of microbes in gut contents might be optimized by cultivation on solidified media. Recent studies indicate that cultivation approaches can resolve better yields of viable microbes than once projected (Felske *et al.,* 1999., Janssen, 2006., Joseph *et al.,* 2003). Many prokaryotic cells in soil display little activity or are in a state of dormancy (e.g., as cysts, starving cells, endospores, or viable but nonculturable cells) (Christensen *et al.,* 1999., Morita, 1993., Stenstrom *et al.,* 2001., van Elsas, 1993). Species of the genera *Aeromonas, Pseudomonas, Sinorhizobium*, and *Ralstonia* form viable but nonculturable cells (Alexander *et al.,* 1999., Edwards, 2000., Grey & Steck, 2001., Manahan & Steck, 1997) and have been detected in the earthworm gut. Revival or conversion to a more active state can occur when conditions become advantageous for growth, and molecules such as glucose and other low-molecularweight- soluble compounds can trigger such

activations (Edwards, 2000., Stenstrom *et al.,* 2001). An activation of viable but nonculturable cells is coincident with an increase in the culturability of such cells (Edwards, 2000). Endospores from bacilli and clostridia are common in soil (da Silva *et al.*, 2003., Garbeva *et al.,* 2003., Ovreas & Torsvik, 1998., Slepecky & Leadbetter, 1984), and their germination is induced by amino acids and sugars (Setlow, 2003), both of which are abundant in the earthworm gut. Indeed, endospores appear to germinate during gut passage (Fischer *et al.,* 1997). Thus, it can be hypothesized that the culturability of certain members of ingested soil microbial biomes increases during gut passage, and that this increase in culturability is attributed to a variety of events, including the stimulation of relatively inactive microbial cells, the activation of viable but nonculturable cells, and the germination of endospores. This hypothesis is supported by various observations. Whereas the culturability (i.e., the ratio of cultured to total microbial cell numbers) of the total microbial load of soil with classic microbiological media may approximate 0.1% (Amann *et al.,* 1995), reported culturabilities of the total microbial load of gut contents are as high as 63% (Pedersen & Hendriksen, 1993). Although culturabilities from midgut contents are higher than those from foregut contents (Fischer *et al.,* 1994), gut passage occurs in 2–24 h and is projected to be too fast for massive growth and replication of ingested microorganisms (Brown & Doube, 2004., Drake *et al.,* 2006). Thus, the paradox that the cultured abundances of microbes in the earthworm gut and preingested soil differ greatly while total cell numbers of these two materials differ only marginally (Karsten & Drake, 1997) may be explained, at least in part, by a metabolic activation that leads to an increased culturability of ingested soil microorganisms. This metabolic activation in the microenvironment of the earthworm gut has also been called a priming effect (Lavelle *et al.,* 1997). Although it must be anticipated that certain members of soil microbial biomes replicate during gut passage and would thus yield increased viable counts of microbes subsequent to gut passage, the

collective information available makes it unlikely that an explosive replication of ingested microorganisms occurs during gut passage.

The burrowing and casting activities of earthworms contribute to the activity of soil micro organisms (Edwards, 1998) and nutrient enriched earthworm casts are good media supporting microbial growth (Lee, 1985). Many authors have studied the microbial community in the gut of earthworms (Fisher *et al.,* 1997., Karsten & Drake, 1997). It is well known that Gram- negative bacteria are common inhabitants of the intestinal canal of earthworms (Reyes, 1976). Number of *Vibro sp.* and *Aeromonas hydrophila* were reported to frequent in the gut of earthworms *Eisenia lucens* (Marialigeti, 1979) and *Pheretima sp.* respectively. Daane *et al.* (1998) found, by scaning electrone microscopy, that there were numerous rod shaped bacteria in egg capsule of *E. fetida*, and suggested a mutualistic association. Edwards (1998) reported that vermicompost is rich in microbial populations and diversity, particularly fungi, bacteria and actinomycetes.

Epigeic earthworm guts preferentially stimulate some microorganisms, and reduce others. Phenomena occurring in other ecological categories, such as digestion of protozoa (Brown, 1995) and fungal hyphae, (Dash *et al.,* 1986) release of antibiotics (Kristufek *et al.,* 1993) and selective ingestion of microflora (Cooke & Luxton, 1980) likely also occur in epigeic earthworm guts, leading to a relative dominance of microorganisms different to that found in uningested soils. For example, various *Vibrio* spp. and *Streptomyces lipmanii* were the dominant bacteria and actinomycetes in *Eisenia lucens* guts (Contreras, 1980., Mariaglieti, 1979) but found in low abundance in its habitat (decomposing wood). Further research with other earthworm species, particularly *in-vitro* descriptions of gut microflora and processes, will help pinpoint the mechanisms by which epigeics differentially stimulate gut microflora species and their activity, and the resulting effects on cast properties and microfloral communities.

In the gut of anecic earthworms, both direct (a few basic enzymes) and indirect (mutualistic) digestion processes are probably present. However, since few studies have described the enzymatic capacities and intestinal mucus production of anecics other species must be tested to confirm this (Lavelle *et al.,*1995).

Contrary to epigeics that ingest only litter, anecics ingest significant proportions of soil ranging from 61% (*Lumbricus herculeus*) up to 90% (*M. lamtoiana*) depending on seasonal activity and litter quality and availability throughout the year (Bouché & Kretzschmar, 1974., Kayondo, 1984). Litters of higher N content or colonized by particular fungi species are preferentially ingested (Abbott & Parker, 1981., Cooke & Luxton, 1980).

Transit through *L. terrestris* guts has shown a differential stimulation or reduction in microbial populations: fungi and active protozoa (not cysts) are generally reduced but then rapidly multiply in casts, while bacteria and actinomycete populations tend to increase in both guts and casts, though this appears to be primarily due to increases in activity and culturability, and not population growth *per se* (Brown, 1995).

Endogeic digestion appears to be primarily mutualistic, with highly variable amounts of intestinal mucus being produced in the foreguts, depending on feeding groups and species (Lavelle *et al.,* 1995). Highest production was observed in poly- and meso-humic endogeics. Gut microflora are also preferentially stimulated or reduced depending on earthworm and microbe species, soil environment, and food ingested (Brown, 1995). Fungal hyphae, active protozoa, algae, myxomycetes and nematodes may be digested, while encysted or protected forms survive gut passage and then rapidly proliferate in casts (Brown, 1995). Cell viability is often positively affected so that higher populations of many microorganisms are detected in casts than bulk soils when using plate counts (CFU's) or other methods (Brown, 1995). Microbial dispersal, such as VAM, *Frankia, Rhizobia* and other beneficial bacteria (e.g., biocontrol species, rhizobacteria), or plant pathogenic fungi and parasitic

nematodes by endogeics is important but often overlooked in soil ecology and plant pathology (Brown, 1995).

Studies (Kale *et al.,* 1991) on incidence of cellulolytic and lignolytic organisms in earthworm worked soils showed that symbiotic microflora of worms are involved in lignin degradation. The total microbial load in the different regions of the gut of worms has also shown more intense colonization of microbes in the anterior part of the intestine than the other region. Bisesi (1990) found that application of earthworm biotechnology in conjunction with indegenous microbial activity, under ambient conditions of temperature and seasonal changes enhance the rate of stabilization and turnover of biological sludge. Earthworm have been shown to selectively consume different type of plant material (Piearce, 1989), and to select different fungal species when offered on filter paper disc (Cooke, 1983). The presence of fungal propagules in the earthworm gut, and in cast material, has been known for some time (Parle, 1963) and earthworm have been implicated in both the reduction and dispersal of soil- borne animal and plant fungal disease and the spread of beneficial group such as mycorrhizal fungi (Gange, 1993). Parle (1963) reported that population of yeast and fungi did not proliferate during passage through the gut, although actinomycetes and bacteria did.

Several recent studies have shown that earthworms can mediate bacterial transport into different environments (Daane *et al.*, 1997). Stephens *et al.* (1993) reported that the transport of beneficial bacteria by earthworms were important to the biological control. Due to these characteristics, earthworms act as vectors for the dispersal of soil microorganisms (Madsen & Alexander, 1982) through lateral and vertical zones, and as bioreactors for certain kinds of microorganisms (Parle, 1963). However, several differences exist between the gut condition of earthworm and soil environment (Egert, 2004) To survive throughout gut passage, bacteria must adapt to the anaerobic and physicochemical gut conditions (Horn, 2003) the lysis of microbes by digestive enzymes secreted by the earthworm(Edwards, 1988) and the inhibition of bacteria by inhibitory substances secreted by other bacteria

(Brown, 1995). Therefore, to facilitate the use of earthworms as vectors for the dispersal of beneficial bacteria, the facultative intestinal microorganisms in earthworms must be characterized.

Denitrification in the earthworm gut is involved in the *in vivo* emission of N_2O by earthworms (Matthies *et al.,* 1999), cultured denitrifiers occur in high numbers in the earthworm gut (Karsten and Drake, 1997), and denitrification can occur in earthworm casts (Elliott *et al.,* 1991., Svensson *et al.,* 1986). Most denitrifiers possess the capacity to both produce and consume N_2O (Conrad, 1996), and the net release of N_2O during denitrification is regulated by various parameters, including pH (Sahrawat & Keeney, 1986), the phase of growth (Baumann *et al.,* 1996), and the concentrations of nitrate and electron donors (Kester *et al.,* 1997). High numbers of other organisms that are capable of producing N_2O (i.e., nitrate-dissimilating and nitrifying bacteria) are also present in the earthworm gut (Ihssen *et al.,* 2003). Production of N_2O by nitrate-dissimilating bacteria is favored in systems that contain high levels of organic carbon, like the rumen or the gastrointestinal tracts of higher animals (Kaspar & Tiedje, 1981., Tiedje, 1988.). Some nitrifiers are able to use nitrate or nitrite as electron acceptors and by using this nitrifier denitrification system can produce N_2O and/or N_2 under oxygen-limited conditions (Freitag *et al.,* 1987., Poth & Focht, 1985).

Material and Method

2. Abundance of Earthworms at Rajshahi University Campus:

2.1 Study Area

Rajshahi University campus was the study area of the present research work. Rajshahi University is located in Rajshahi city, a North-Western city of Bangladesh. Geographically it is located on 24°22'14" North latitude and 88°38'6" East longitude. The university's main campus is located in Motihar thana, on the eastern side of the city of Rajshahi and a mile from the river Padma. The campus area is nearly 753 acres (3.05 km²). There are different types of habitates for earthworm in Rajshahi University campus such as shady land, flower garden, vegetable garden, crop land, drainage of residential hall etc. In present research work three types of habitates i.e. shady land, crop land and drainage of residential hall were selected for study.

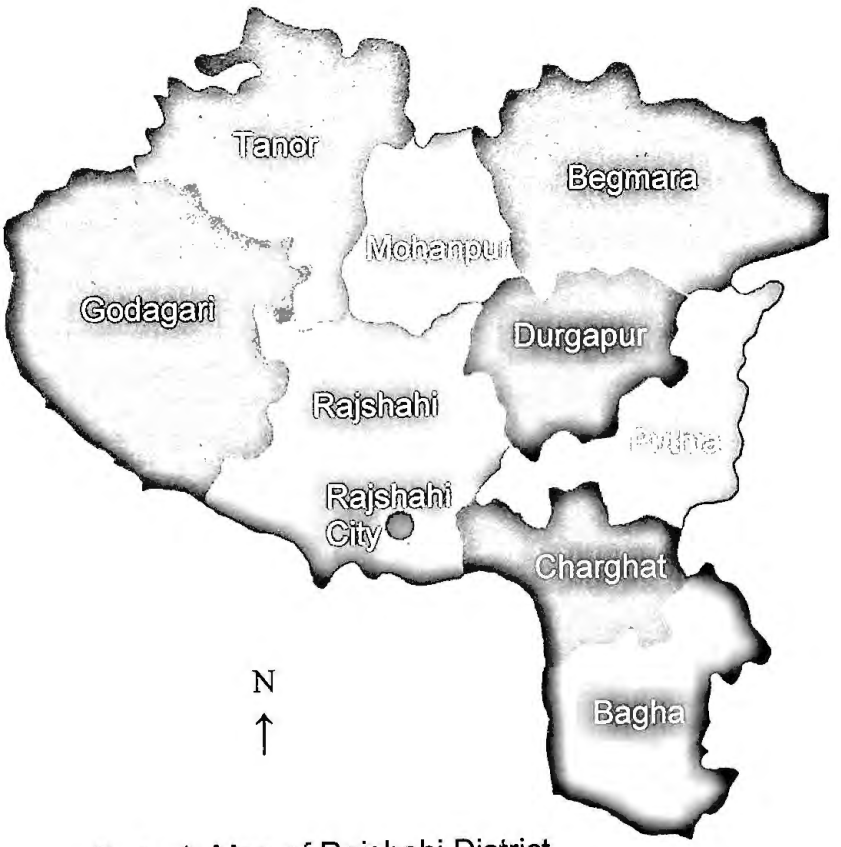


Plate 1: Map of Rajshahi District

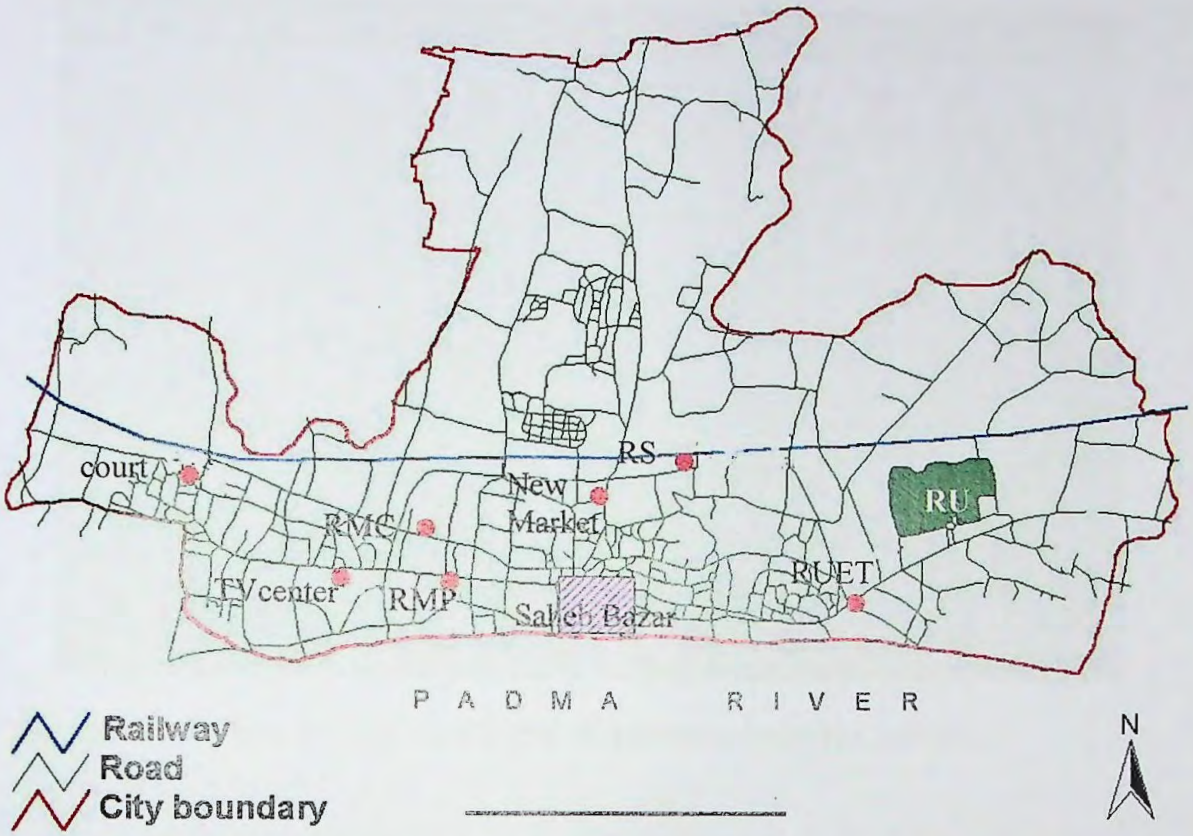


Plate 2: Map of Rajshahi City (RMC= Rajshahi Medical College, RMP= Rajshahi Metropolitan Police, RS= Rajshahi Station, RU= Rajshahi University)

Plate 3: Shady land in the Rajshahi University Campus

Plate 4: Crop land in the Rajshahi University Campus

Plate 5: Drainage of residential hall in the Rajshahi University Campus

2.2 Collection and Counting of Earthworm

Crop land, shady land and drainage area of residential hall of Rajshahi University campus were selected for earthworm faunal survey from September, 2004 to August, 2005. The fortnightly sampling dates were fixed on 14th day for first sampling and 28th day for 2nd sampling each month. Quadrate sampling method was used in this study where 20cm×20cm×20cm quadrate was used because this size of quadrate is the most suitable and efficient for earthworm extraction (Mukharjee and Singh, 1986). In each sampling day quadrate was used in triplicate for collecting earthworm. Thus, total 0.048 cubic meter (0.2m×0.2m×0.2m =0.008m^3×3=0.024m^3×2=0.048m^3) area of each habitates were studied each month for earthworm collection. Hand sorting method Lewis & Taylor's (1979) was followed to collect earthworm. The soil sample was hand sorted carefully in the field and the number of worms found in each sample was recorded. Earthworms of different soil samples were collected in labeled plastic containers and carried to the laboratory for their species identification, population counting and finally for morphohistological and microbiological study.

2.3 Identification of Earthworm

Collected earthworms were identified with an identification key. Current complete key can be found in Gates (1972), Julka (1988) and Reynold *et al.* (1995). In the present study an identification key modified for identification of earthworms found in north-western region of Bangladesh was used (Pramanic, 2001).

Identification key of earthworms found in north-western region of Bangladesh (Pramanic, 2001).

1. Testes and male funnels intraseptal, male pores at or near 10/11, clitellum single-cell thick in X-XII.	**2** (Fam. **Moniligastridae)**
Testes and male funnel interseptal, male pores posterior to 10/11, clitellum multiple layers of cells in XIII and posteriad segments.	**3**
2. Genital markings usually close to spermathecal pores.	*Drawida limella*
Genital markings usually close to male porophores.	*Drawida nepalensis*
3. Male and spermathecal pores indistinct.	**4** (Fam. **Almidae & Glossoscolecidae**)
Male and spermathecal pores distinct.	**5**
4. Body quadrangular, wall protuberant at maturity as a longitudinal lamellar ridge (wing) through several clitellar segments.	*Glyphidrilus tuberosus* (Fam. **Almidae**)
Body cylindrical, wall without ring, setae quincunx, at least in caudal region.	*Pontoscolex corethrurus* (Fam. **Glossoscolecidae**)
5. Setae perichaetine, prostate racemose or lobular.	**6** (Fam. **Megascolecidae**)
Setae lumbricine, prostate tubular.	**17** (Fam. **Octochaetidae**)

6. Intestinal caeca absent.	**7**
Intestinal caeca present.	**12**
7. Genital marking absent.	**8**
Genital marking present.	*Polypheretima elongata*
8. Sexthecal, male and spermathecal pores situated apart from midline.	*Lampito mauritii*
Quadrithecal, male and spermathecal pores approximated very close to the midline.	**9**
9. Last pair of hearts in XII, penial setae present.	*Perionyx exacavatus*
Last pair of hearts in XIII, penial setae none.	**10**
10. Penes distally bifid and in a median vestibulum.	*Perionyx horai*
Penes distally not bifid nor in vestibulum.	**11**
11. Penes in paired invaginations, body segments uniannular.	*Perionyx modestus*
Penes in a single transverse invaginations, body segments biannular.	*Perionyx simlaensis*
12. Male pores superficial, not invaginated.	**13**
Male pores invaginated.	**14**

13. Male pores not in distinctly demarkated porophores but in depressions, spermathecal diverticulum ends in a knob like seminal chamber.	*Amynthas alexandri*
Male pores not in depression but on distinctly demarkated porophores, spermathecal diverticulum ends in an elongate moniliform seminal chambers.	*Amynthas diffringens*
14. Spermathecae 2 pairs, pores in 6/7 to 7/8.	*Metaphire planata*
Spermathecae more than 2 pairs.	**15**
15. Spermathecae 3 pairs, pores in 6/7 to 8/9.	**16**
Spermathecae 4 pairs, pores in 5/6 to 8/9.	*Metaphire posthuma*
16. Spermathecae with a long diverticulum and a staked accessory gland.	*Metaphire houlleti*
Spermathecae with a long tubular diverticulum but without accessory gland.	*Metaphire umbraticola*
17. Esophagus with more than one gizzards.	**18**
Esophagus with single gizzard.	**19**
18. Biprostatic.	*Dichogaster saliens*
Quadriprostatic.	*Dichogaster modigliaii*
19. Biprostatic.	**20**
Quadriprostatic.	*Octochaetona beatrix*

20. Holandric, seminal vesicles in IX and XII.	**21**
Metandric, seminal vesicles only in XII.	**22**
21. Polydiverticulate, without penial setae, seminal vesicles of XII extend much posteriorly upto about XXIV.	*Eutyphoeus quadripapillatus*
Polydiverticulate, with penial setae, seminal vesicles not so extended.	*Eutyphoeus incommodus*
22. Avestibulate.	**23**
Vestibulate.	**24**
23. Lateral intestinal caeca present in XXVIII, female pore single, genital markings paired and unpaired.	*Eutyphoeus gigas*
Lateral intestinal caeca absent, genital markings unpaired, intersegmental on 15/16.	*Eutyphoeus scutarius*
24. Univestibulate, genital marking large, unpaired, spermathecal pores at median half of bc.	*Eutyphoeus gammiei*
Bivestibulate, genital marking small, paired.	**25**
25. Penes annular; genital marking present on XV, usually also on XVI and posteriors to male pores.	*Eutyphoeus orientalis*
Penes elongate; genital marking paired across 15/16 only.	*Eutyphoeus nicholsoni*

2.4 Weather of Rajshahi city:

Data on different components of weather of Rajshahi city i.e. rainfall, relative humidity, minimum and maximum temperature, soil temperature and sunshine hour were collected in each month from September, 2004 to August, 2005. These data on weather were collected from Regional Weather Office, Government of Bangladesh, Rajshahi. Collected data were analyzed by SPSS software for various statistical interpretations.

2.5 Morphohistology of gut of Earthworm:

For morphological study, earthworms were dissected and cleaned to separate the gut. Before dissection, earthworms were anaesthetized with 70% ethyl alcohol. Each specimen to be dissected was washed in sterile distilled water, placed across the second, third and fourth fingers of the left hand (gloved) with the anterior end pointing forward. The fine edge of a flamed pair of dissecting scissors was inserted into the ventral surface at the region of the clitella and with the body wall slightly raised up with the scissors; an incision was made longitudinally along the earthworm. Sterilized dissecting pins were used to hold the earthworm down on a board, stretching out the body wall to expose the internal structures. The gut was then freed from surrounding blood vessels and nephridia with a flamed forceps. Characters of gut of different earthworms were studied and recorded.

After morphological study, gizzard, stomach and intestine of the gut of earthworms were separated and used to prepare permanent slides for histological study of their gut. Permanent slides preparation procedure was as follow:

The different parts of the gut i.e. gizzard, stomach and intestine were fixed in fixative (aqueous Bouin's fluid) for about 18 hours. The fixed tissues were washed in tap water to remove the Bouin's fluid. After washing in water the tissues were dehydrated through different ascending grades of alcohol (50%,

70%, 90%, 95% and 100% at about 5-15 minutes), then passed through the xylene (about 10-15 minutes) and super saturated solution of wax and xylene (at about 10 minutes) for impregration and finally in the melted wax (about 58°C-60°C temperature) for 10 minutes in the incubator for embedding. After this the blocks (into the paper boat) were prepared. The process of trimming was followed and the blocks were prepared in suitable size. Then the block was set in the holder and serial sections of the tissue were cut at 6μ thickness with the help of Rocking Microtome Machine.

The ribbons of the sections put in a petridish which contains water and a few drops of Mayer's albumen solution. Then, the ribbons were stretched on the slide gently in warm condition. The species of the ribbons were set in rows serially. After stretching, the slides were kept for about 12 hours to dry up the excess water from the ribbons.

The sections were deparaffinized by xylene for about 15 minutes and gradually passed through the descending grades of alcohols (100%, 95%, 90%, 75%, 70% and 50 at about 5-15 minutes). Then the sections were stained with the haematoxylene for nuclear staining. Then, the slides were kept in the running tap water for more than 30 minutes to remove the excess haematoxylene.

After this, the slides were counter stained with alcoholic eosin for about 5-10 minutes. After that the sections were dehydrated through different grades of alcohol (70%, 90%, 95% and 100%). Then the tissues were passed through xylene. Finally they were mounted on the slide with cover-slip and Canada balsam. Thus, a permanent histological slide was prepared.

The prepared slides were studied with the help of advanced biological microscope (Motic Bi series). Then, microphotographs of the slides were taken with the help of motic image plus software in Machentosh operating system/ apple computer.

2.6 Bacterial load of gut of Earthworm

Earthworms collected from Rajshahi University Campus were brought to the laboratory in sterile flasks. Earthworms were thoroughly washed thrice with sterile distilled water. Gizzard, stomach and intestine of gut were separated after dissecting the earthworm with sterilized scissors and were washed in distilled water. Then, bacterial load of samples i.e. gizzard, stomach and intestine were measured by serial dilution and by spread plate method (Tortora *et al.* 1998).

Gizzard, stomach and intestine were crushed separately with mortar and pestle. Then, 1gm of each preparation was taken separately in test tubes. Sterile distilled water was added to each test tube to make the volume of each preparation 10ml. Preparations were homogenized. 1ml subsample was taken from each of the above homogenized preparations and added separately in different test tubes containing 9ml sterile distilled water to make 10-fold serial dilutions of each preparation. Preparations were homogenized in every steps of serial dilution. Serial dilutions were made of up to 10^9 dilutions. A 0.1ml aliquot of the sixth (10^6), seventh (10^7), eighth (10^8) and ninth (10^9) dilutions were each inoculated in triplicates by the spread plate technique on nutrient agar plate. Then, inoculated petriplate were incubated at 37°c for 24 hours. Bacterial colonies were counted and recorded after 24 hours of incubation.

Finally, Recorded data were analyzed by SPSS software for various statistical interpretations.

2.7 Pure culture preparation

Bacterial pure culture was prepared by streak plate method (Tortora *et al.* 1998). The typical well isolated colonies were picked up from petriplate and suspended in sterile physiological saline in a test tube and streaked on nutrient agar plates for purification. Streaked plates were incubated at 37°c for 24 hours. After incubation a well isolated typical colony, from each plate was picked up and sub-cultured on nutrient agar slants for maintenance and further studies.

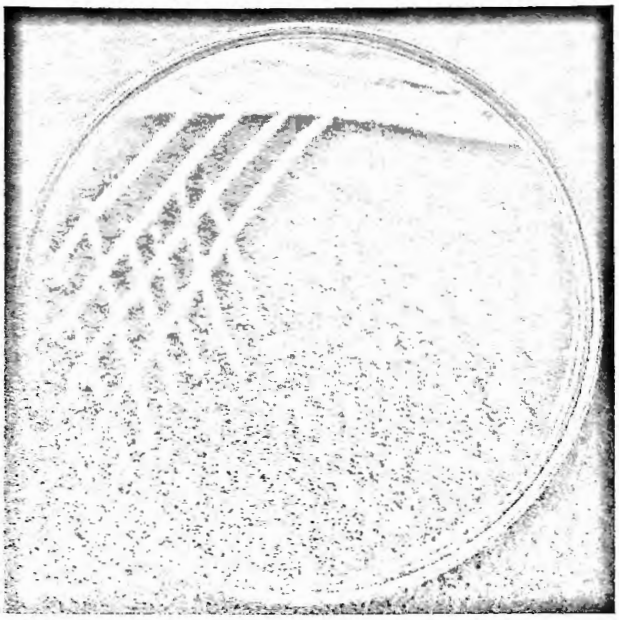


Plate 6: Pure culture of sample bacteria

2.8 Identification of bacteria of earthworm gut

Pure culture of bacteria isolated from the different parts of gut of earthworms were tested for Gram character, morphology, motility, catalase and oxidase reactions, citrate utilization and coagulase test (Collins *et al.,* 1995., Harrigan and McCance, 1976., Seeley and Van Demark, 1972). The isolates were then identified with Bergey's manual of systematic bacteriology.

2.8.1 Gram characters and morphology of bacteria

Gram characters of bacteria isolated from earthworm were identified by Gram staining method (Tortora *et al.* 1998). Bacterial cells collected from colony of pure culture were smeared on a slide to form a thin bacterial film. Then, cells were fixed with mild heat. The slide with fixed bacterial film was flooded with crystal violet solution for 30 second and washed thoroughly with gentle

stream of tap water. The slide was flooded with iodine solution for 1 minute and washed thoroughly with gentle stream of tap water. The slide was then washed with alcohol (95%) for 10-15 second. Alcohol was washed thoroughly with gentle stream of tap water. The slide was covered with safranin (counter stain) for 1 minute. Finally, slide was washed with tap water and dried. Prepared slide was then examined under microscope for study of gram characters and morphology of bacteria.

2.8.2 Motility test

Motility of bacteria was tested by hanging drop method (Collins *et al.*, 1994). A small drop of liquid bacterial culture was placed in the centre of a coverslip. A small drop of water was also placed at each corner of the coverslip. A slide with a central depression was inverted over the coverslip. The coverslip was attached to the slide and when the slide was inverted the drop of bacterial culture was suspended in the well. Then, the slide was examined microscopically (x400) for motility of organisms. A darting, zig-zag, tumbling or other organized movement indicate positive result. On the other hand no movement or Brownian motion only indicates the negative result.

2.8.3 Catalase test

This test was done to detect the presence of the enzyme catalase in sample bacteria. It catalyses the breakdown of hydrogen peroxide (H_2O_2) with the release of free Oxygen. Catalase is found in most aerobic and facultative anaerobic bacteria. Catalase is not found in anaerobes. In this test a capillary tube was dipped into 3% H_2O_2. Then, a bacterial colony was touched with that capillary tube. The tube was observed for bubble indicating a positive reaction. It is important not to contaminate the bacterial colony under test with blood agar. Red blood cells contain catalase and their presence will give a

false positive result. Old cultures may loose their catalase activity, possible resulting in a false negative result.

2.8.4 Oxidase test

This test was done to determine the presence of oxidase enzymes. This test is useful in differentiation of Enterobacteriaceae and Pseudomonas. The Oxidase test paper contains tetramethyl-p-phenylenediamine which serves as an alternate substrate for the cytochrome oxidase reaction. In the reduced state the test paper is colourless but when oxidized it become purple. In this test a piece of the test paper was held with forceps and touched onto an area of heavy growth. Colour of the test paper change to purple within 10 seconds indicating positive result. The test is only reliable as long as the time limit for a positive result is adhered to (up to 60 seconds maximum).

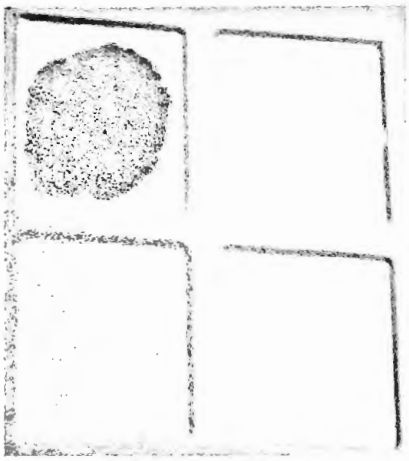


Plate 7: Oxidase test paper showing positive result

2.8.5 Citrate utilization test

Citrate utilization test was carried out to determine if an organism is capable of utilizing citrate as sole carbon source for metabolism with resulting alkalinity. In this test a Simmon's Citrate agar tube was streaked with the organism and incubated at 37° C for 48 hours. The medium must be lightly inoculated (from plate cultures, not from a broth) to avoid a carry over of

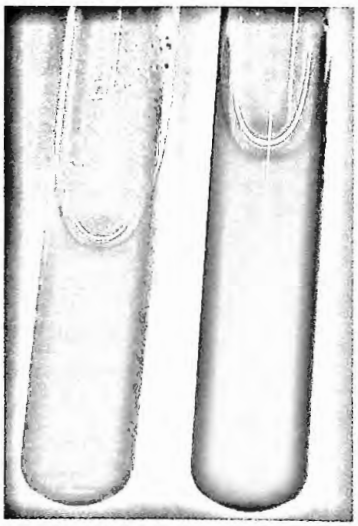


Plate 8: Citrate utilization test

nutrients, which may lead to a false positive result. After incubation, tube was examined for change of medium from its initial green colour to deep blue indicating positive result. Growth on the medium is accompanied by a rise in pH to change the medium from its initial green colour to deep blue. This characteristic can used to differentiate members of the family Enterobacteriaceae and other gram-negative rods.

2.8.6 Coagulase test

Coagualse test is performed to test the ability of the microorganism to clot plasma by the action of coagulase. Rabbit plasma was taken in a test tube and inoculated with one single colony. Colony was broken up and stirred until blended in plasma. Then, it was incubated at 37° C for 24 hours. If bacteria isolated from gut were able to clot blood, then they were coagulase positive.

Result

3.1 Abundance of earthworm at Rajshahi University Campus

Six species of earthworm identified from three different habitats i.e. shady land, cropland and beside the drainage of residential hall at Rajshahi University Campus. These are:

1. *Metaphire posthuma*,
2. *Eutyphoeus orientalis*,
3. *Eutyphoeus nicholsoni*,
4. *Eutyphoeus incommodus*,
5. *Lampito mauritii* and
6. *Drawida limella*.

1. *Metaphire posthuma*

Length 75 to 135 mm; diameter about 3 to 6mm, total segments 95 to 125. Colour light to dark grey or brown. Prostomium small, epilobous, tongue short, usually open. First dorsal pore mainly on 12/13. Clitellum ring shaped, distinct. Male pores minute on xviii. Female pore minute single medium, presetal on xiv. Body cylindrical, elongated but become coiled like spring when disturbed. Segment ii-viii biannular, ix-xiii tetraannular, post clitellars triannular but anal segments biannular. Genital markings as small circular, papillae generally on setal arcs of xvii and xix, slightly internal to the line of male pores.

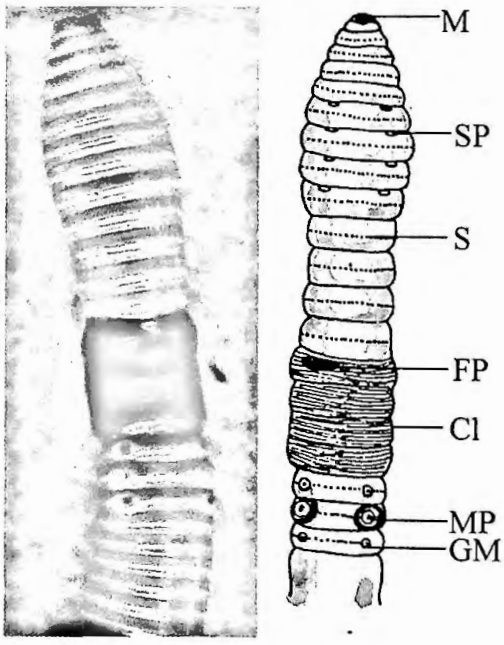


Plate 9: *Metaphire posthuma*

2. *Eutyphoeus orientalis*

Length 131 to 270 mm, diameter 4 to 8 mm, total segments 158 to 234. Colour violet brown dorsally, with darker middorsal stripe; grayish laterally and ventrally. Prostomium combined pro/tanylobic, tongue border parallel. First dorsal pore at 11/12, often 12/13. Setae lumbricine, all ventral, closely paired. Clitellum ringed, on xiii or ½ xiii-xvii. Male pores on xvii, discharge into paired hollow vestibula opening on to the body surface through circular to oval or slit like aperture at mid bc; some times the male field presents a pair of bracket shaped grooves each overhung on its outer side by a thickened ridge, and the pores in the posterior corner of each bracket, a little outside of *b*. Female pore single, presental at *a*, on the left side of xiv. Spermathecal pores minute transverse slits at *bc* on 7/8. Body elongated, cylindrical, large, heavier than other worms. First three segments simple four to six bi-annular seven to pre-clitellers multi annular. Genital markings mostly paired postsetal on xv, xvi, inter-segmental usually on 18/19 to 20/21 or often up to 26/27, at *ab*.

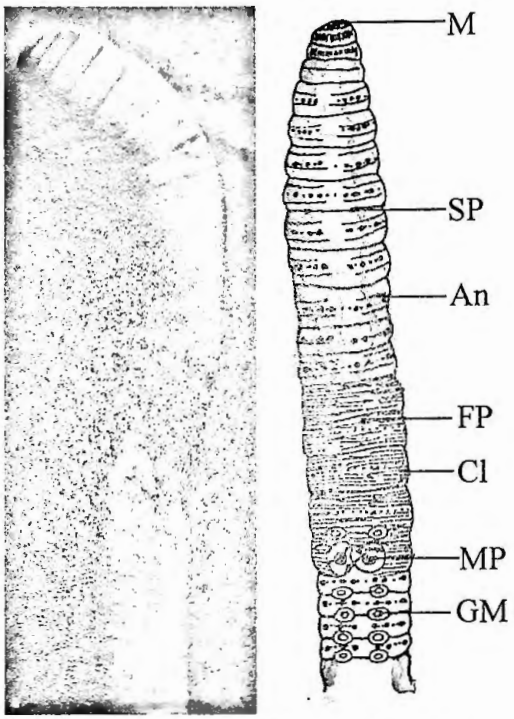


Plate 10: *Eutyphoeus orientalis*

3. *Eutyphoeus nicholsoni*

Length 112 to 210 mm, diameter 4 to 7 mm, total segments 113 to 205. Colour violet-grey or brownish dorsally, yellowish grey ventrally. Prostomium prolobous or combined protanylobous. First dorsal pore on 12/13. Setae

lumbricine, all ventral. Clitellum ring shaped, on ½ xiii-xvii, male field included. Male pores slit like discharge into paired, deep vestibula surrounded by a common ridge opening onto the surface at *ab*. Female pore paired on xiv presetal at a. Bithecal, spermathecal pores small, transverse slits on 7/8 at a. Body elongated cylindrical, iv-vi are trianular, pre-clitellers tetraannular, post-clitellers mostly trianular with a few biannular segments posteriorly. Genital markings closely paired, circular on 15/16, surrounded by a common wall, separated from each other in the middle line by a groove.

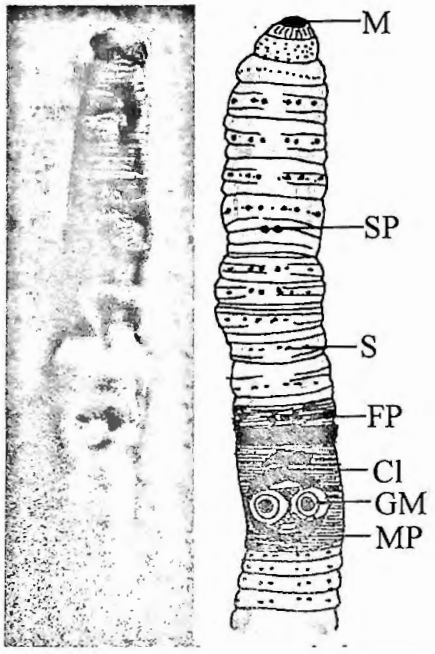


Plate 11: *Eutyphoeus nicholsoni*

4. *Eutyphoeus incommodus*

Length 67 to 112 mm, diameter 3 to 5 mm, segments 110 to 164. Colour browish olive to colour less. Prostomium combined proepilobic or protanylobic. First dorsal pores started from 11/12, sometimes 10/11 or 12/13. Setae lumbricine, all ventral. Clitellum almost annular but much less marked ventrally, includes $^{1}/_{2}$xiii-xvii. Male pores, on xviii, within slight transversely placed fissures, at or close to *b*, each at the centre of a disc-shaped to slightly concial porophore, avestibulate. Female pores paired, presetal at or slightly median to *a*, on xiv. Spermathecal pores paired small transverse slits, in 7/8, slightly lateral to *b*. Body cylindrical, elongated. First three to four

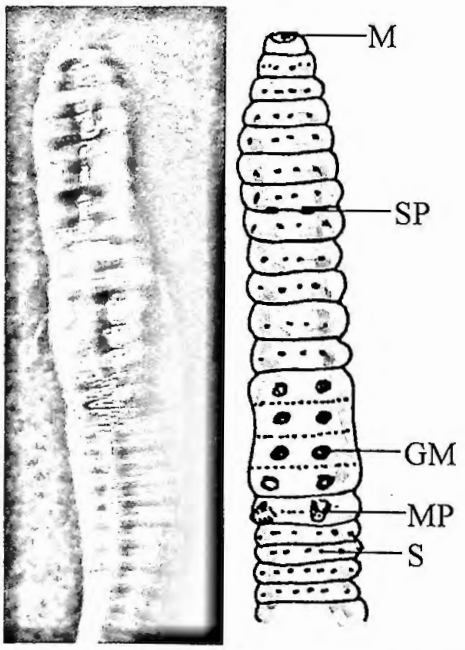


Plate 12: *Eutyphoeus incommodus*

segments simple next three to four biannular, rest of pre-cliteller segments triannular and so also those behind clitellum. Genital marking paired, post setal on xiii, xiv-xvi (4 or 3 pairs), at *ab*, almost circular in outlines.

5. *Lampito mauritii*

Length 72 to 152 mm, diameter 3.5 to 5.5mm, total segments 126 to 179. Colour in dorsum greyish, brownish or yellowish with purplish tinge at anterior end. Prostomium, epilobic. First dorsal pore on 10/11 or 11/12. Preclitellers with 40 to 50, post cliltellers with 30 to 44. Clitellum annular, xiv-xvii. Male pores paired superficial on xviii. The female pores minute, approximated in very close to the middle line, on xiv. No genital marking.

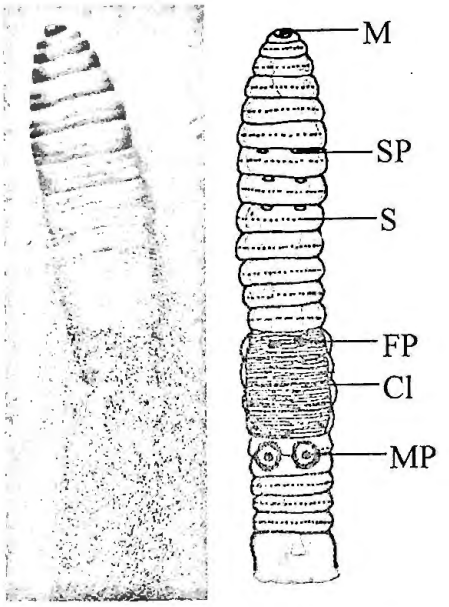


Plate 13: *Lampito mauritii*

6. *Drawida limella*

Length 56 to 89 mm, diameter 3 to 5 mm, total segments 115 to 175. Colour yellowish grey or non-pigmented. Prostomium prolobic. Dorsal pore lacking. Setae lumbricine, closely paired. Clitellum ring shaped, indistinctly on x-xiii. Male pores obvious on transverse oval porophores at 10/11, midway between b and c. Female pores at b. Spermathecal pores at 7/8, as small, transverse slits, just ventral from c. Genital markings small on male porophores and often as broad transversely oval mid-ventral cushions on vii-xi.

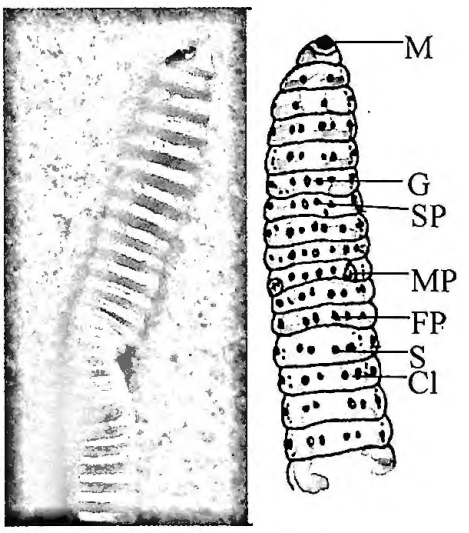


Plate 14: *Drawida limella*

Abundance of earthworm in three studied habitats from September 2004 to August 2005 was presented below in Figure 1, 2 and 3.

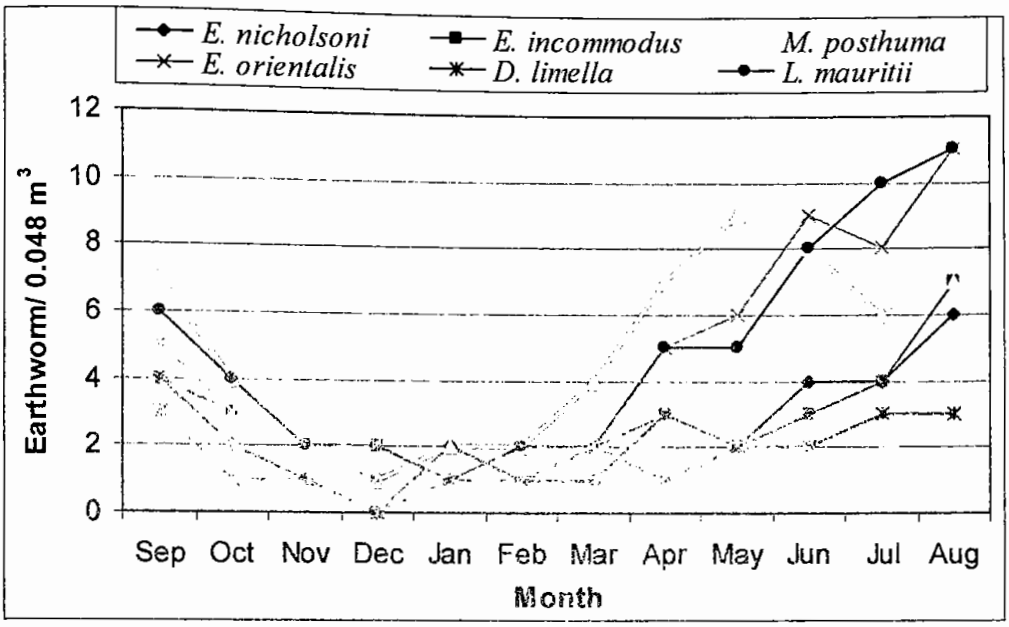


Figure 1: Abundance of earthworms in Shady land in different month

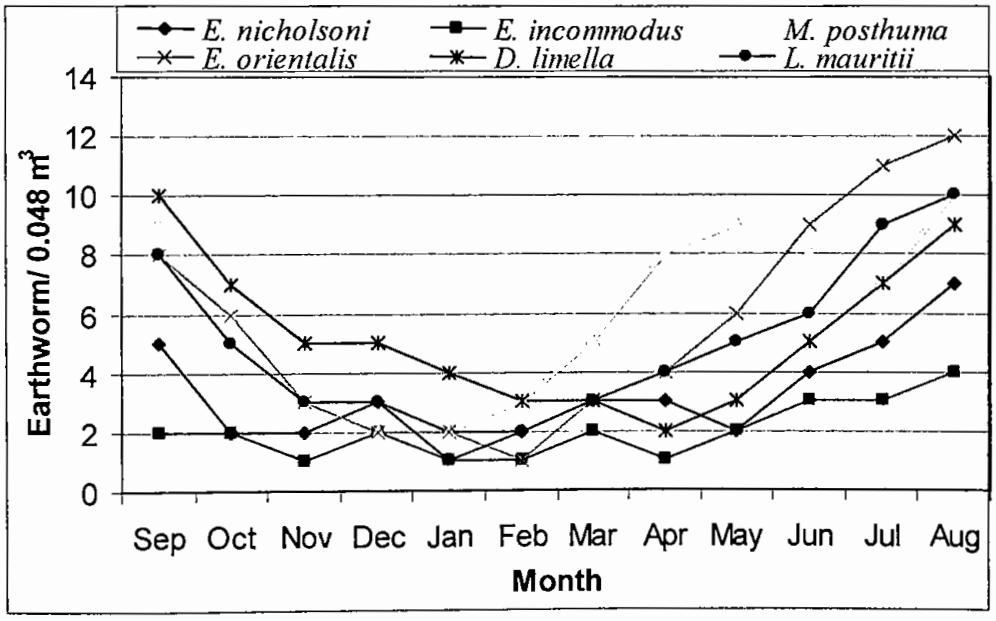


Figure 2: Abundance of earthworm in cropland in different months

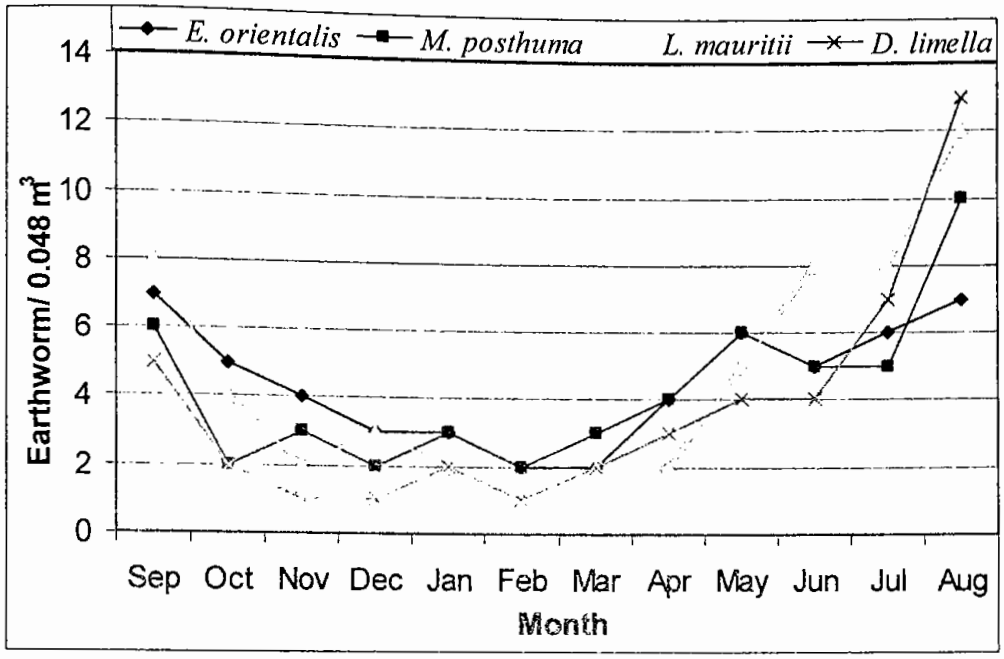


Figure 3: Abundance of earthworm beside drainage of Residential hall area in different months

The Figure 1, 2 and 3 show that earthworm diversity was present in the studied habitats. All of the six identified species of earthworm were present in both shady land and cropland. But, *E. nicholsoni* and *E. incommodus* were absent in habitat beside the drainage of residential hall. The Figures also shows that the number of earthworms varied with month. In most cases, higher number of earthworm was recorded on July, August and September. On the other hand, lower number of earthworm was recorded from November to April. The lowest or zero population was recorded on December 2004 for *E. nicholsoni* and *D. limella* in shady land while the highest population (13 worms/ 0.048 m^3) was recorded on August 2005 for *D. limella* in habitat beside drainage of Residential hall.

3.2 Weather of Rajshahi city:

The figure 4 shows that in Rajshahi city the lowest average temperature (Minimum 11.3°C, Maximum 23.8°C and Soil 18°C) was recorded in January, 2005. The highest average temperature of soil (32.93°C) was recorded in

June, 2005. Average rainfall was the highest (16.44 cm) in July, 2005, and the lowest (0.0 cm) in November and December, 2004. Average sunshine hour and relative humidity were the highest in September, 2004 and the lowest in November, 2004 and in February, 2005 respectively.

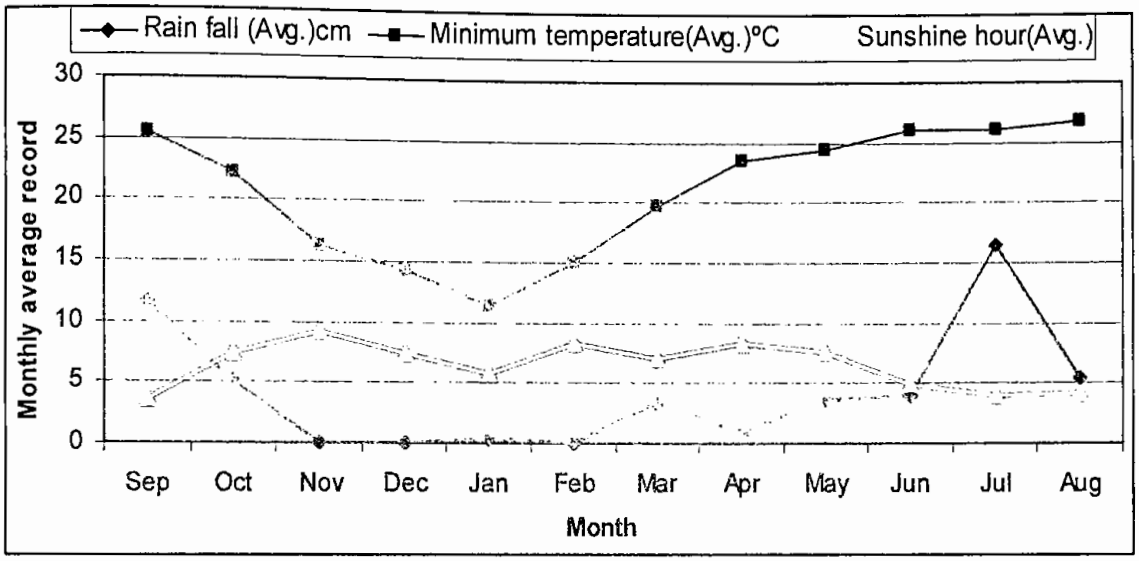


Figure 4 (a): Weather chart of Rajshahi city (September, 2004- August, 2005)

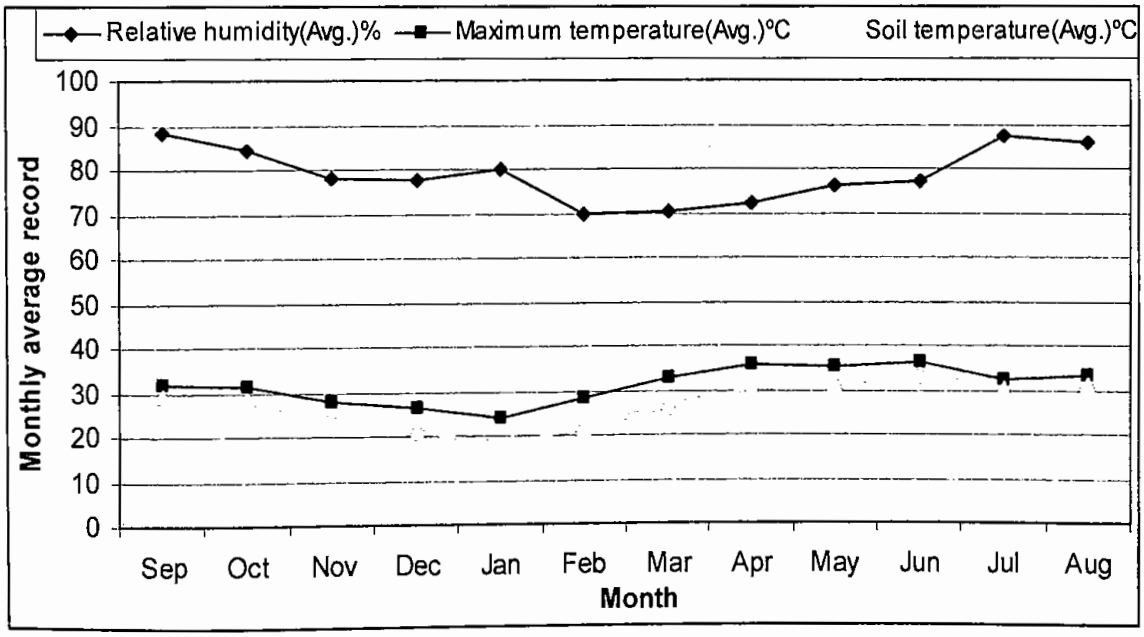


Figure 4(b): Weather chart of Rajshahi city (September, 2004- August, 2005)

3.3 Morphohistological study of gut of earthworm

The gut of earthworm is a long and straight tube of varying diameter, which runs through the entire length of the body from the mouth at the anterior end to the anus at the posterior end. It is held in position by the intersegmental septa. It is functionally regionated into various parts which are mouth, buccal chamber, pharynx, oesophagus, gizzard, stomach, intestine and anus.

The eosophagus terminates into a prominent, oval, hard, thick walled and highly muscular organ, called gizzard. More than two, generally three gizzards is found in *D. limella* while single large gizzard is found in remaining five studied species of earthworm. In *D. limella* gizzards are segmentally arranged in 12^{th} -15^{th} or 12^{th} -14^{th} segments. Gizzard is present on 7^{th} segment in *E. orientalis* and *E. nicholsoni* while that is in the gap in *E. incommodus*. In *M. posthuma* large single gizzard is present in 8^{th} segment but in *L. mauritii* gizzard is found in 5^{th} or often in 6^{th} segment.

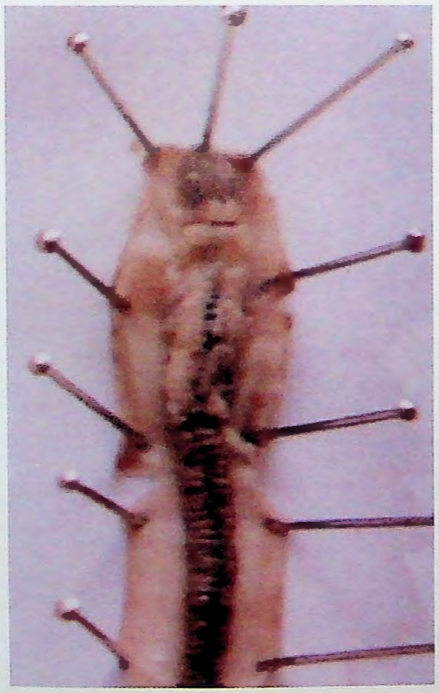
In *E. incommodus* calciferous gland is present in 7^{th} segment extending into 11^{th} segment also. In *E. nicholsoni* one pair calciferous gland is present on 12^{th} segment. Calciferous glands in *E. orientalis* is as usual in metandric species. Calciferous gland is absent in *L. mauritii*.

The gizzard is followed by a short narrow tube, the stomach, which extend from segments 8^{th} to 14^{th} in *E. nicholsoni,* 9^{th} to 12^{th} or 13^{th} in *E. incommodus,* 8^{th} to 14^{th} or 15^{th} in *E. orientalis,* 14^{th} or 15^{th} to 15^{th} or 16^{th} in *D. limella,* 7^{th} to 14^{th} in *L. mauritii* and 9^{th} to 14^{th} in *M. posthuma*. Stomach has sphincter at each end. The wall of stomach is highly vascular and glandular and thrown into internal fold. Stomach is the longest in *L. mauritii* and *E. orientalis* while that is the shortest in *D. limella*.

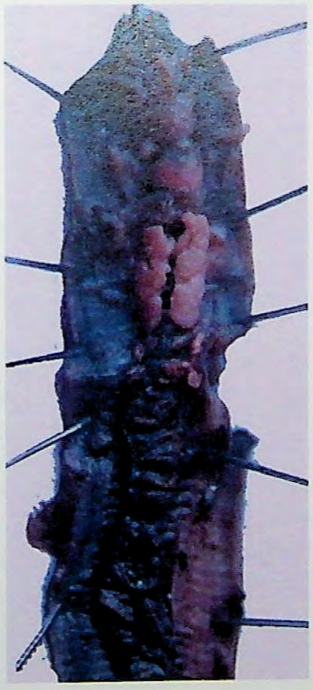
Metaphire posthuma

Eutyphoeus orientalis

Eutyphoeus nicholsoni



Eutyphoeus incommodus



Lampito mauritii

Drawida limella

Plate 15: Morphology of gut of earthworm of Rajshahi University campus

The region next to the stomach is the intestine, which is a longe, wide and thin walled tube. Intestine starts from segment 15th in *E. nicholsoni,* 13th or 14th in *E. incommodus,* 15th or 16th in *E. orientalis,* 16th or 17th in *D. limella,* 15th in *L. mauritii* and *M. posthuma.* Lateral intestinal caecum is absent in *E. incommodus, E. orientalis* and *E. nicholsoni* but numerous midventral caeca started from 35th segment in only *E. nicholsoni.* On the other hand, pared and simple lateral caeca is present in *M. posthuma.* Typhlosole is insignificant or rudimentary in *L. mauritii* while typhlosole is simple and lamelliform in *M. posthuma.*

Permanent histological slides of gizzard, stomach and intestine of earthworms were prepared and studied. Photographs of these slides are shown below in plate 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32 and 33.

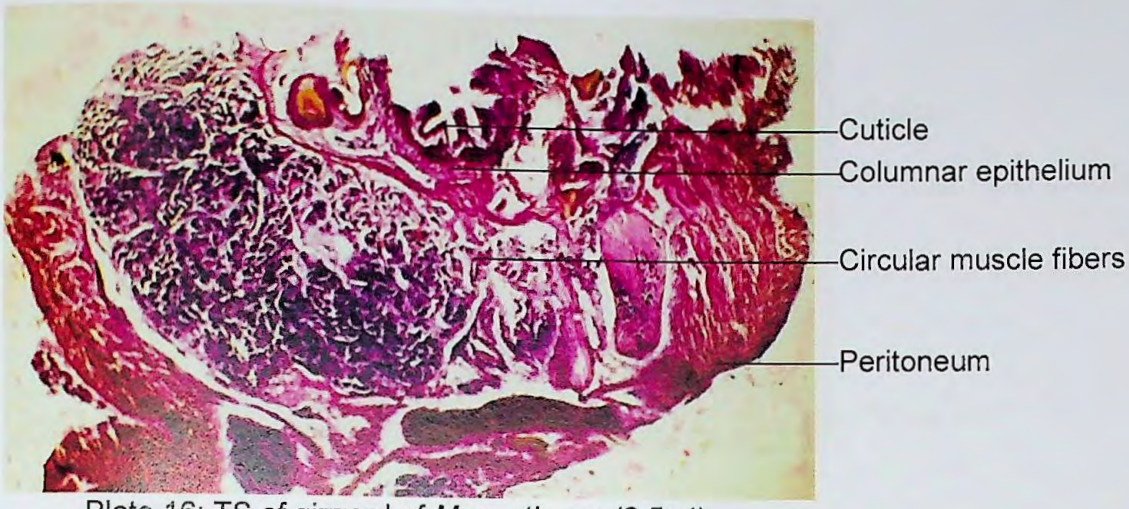


Plate 16: TS of gizzard of *M. posthuma* (2.5×4)

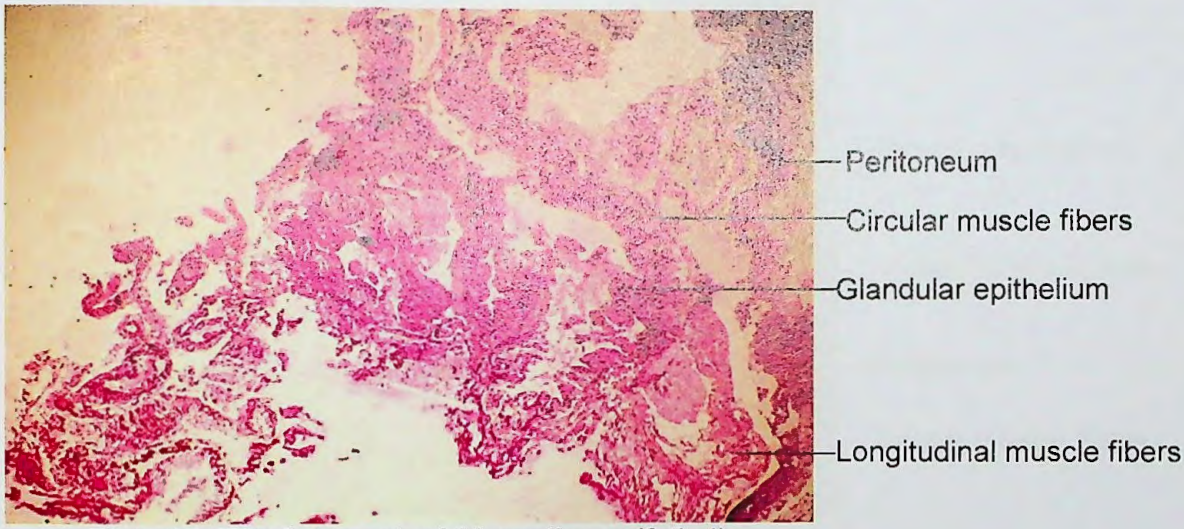


Plate 17: TS of stomach of *M. posthuma* (2.5×4)

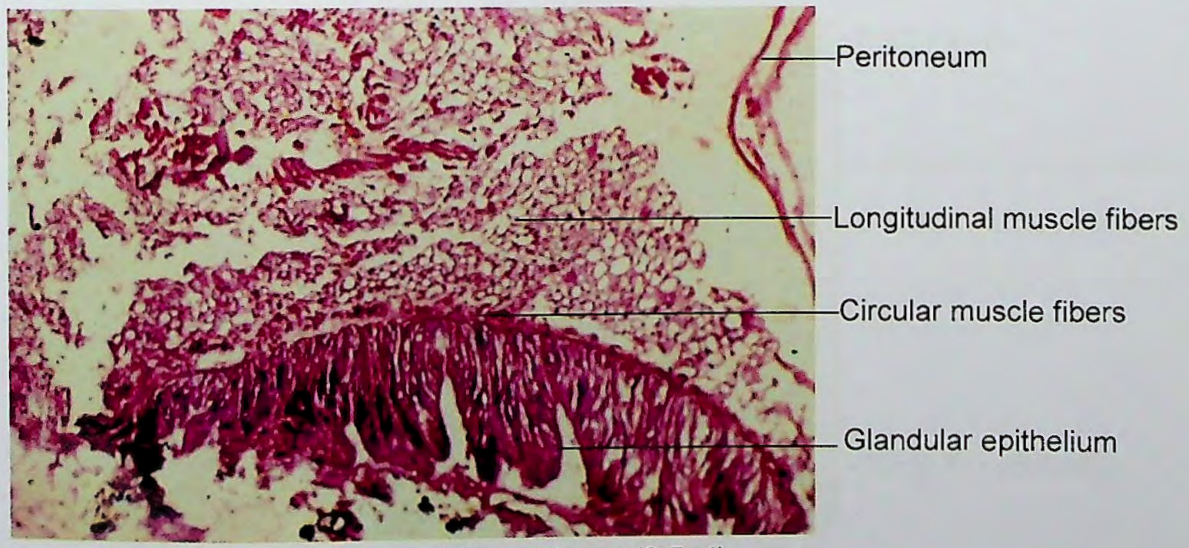


Plate 18: TS of intestine of *M. posthuma* (2.5×4)

Plate 19: TS of gizzard of *E. orientalis* (2.5×10)

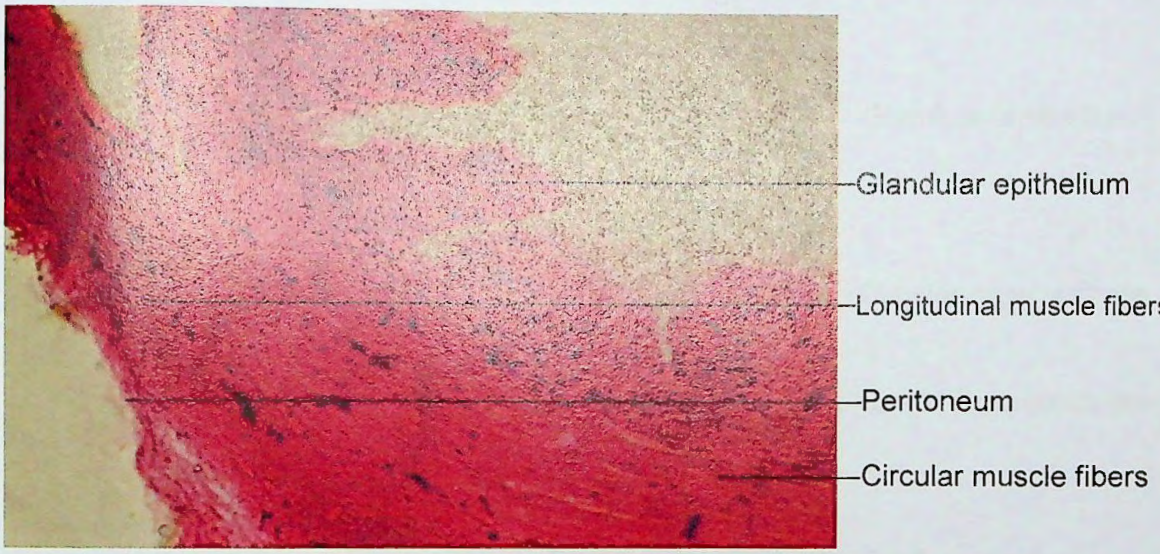


Plate 20: TS of stomach of *E. orientalis* (2.5×20)

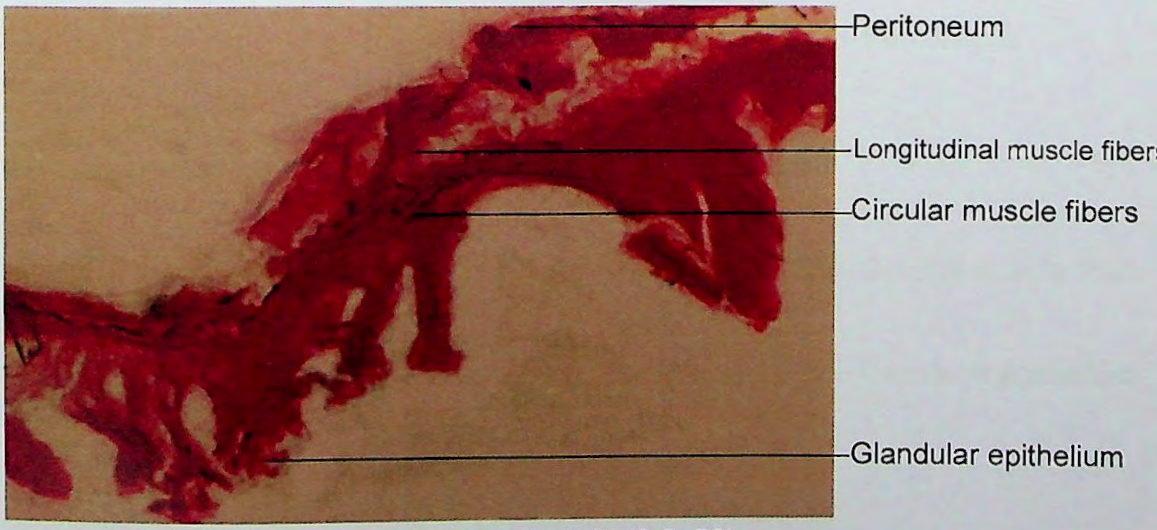


Plate 21: TS of intestine of *E. orientalis* (2.5×20)

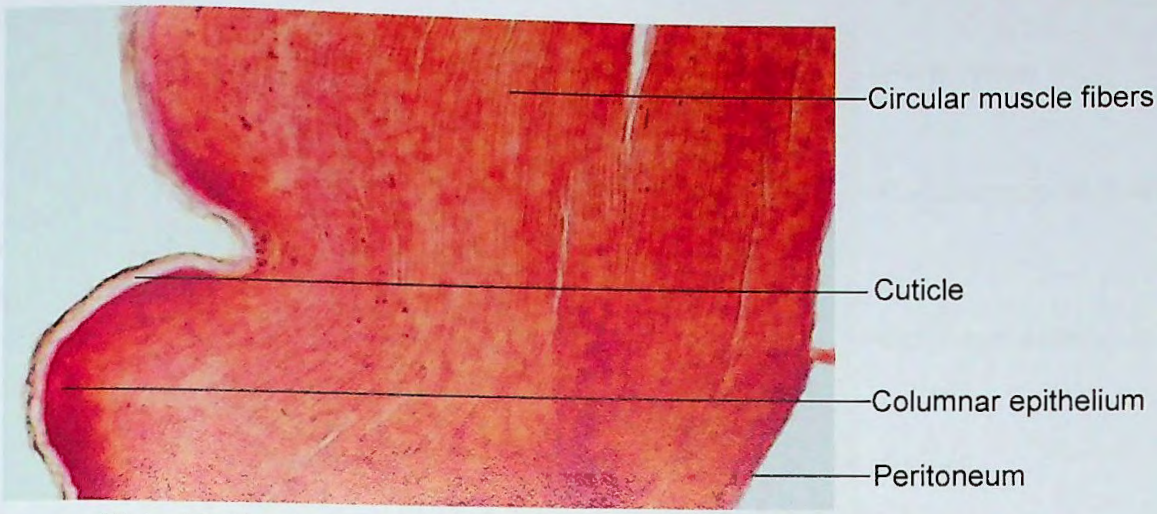


Plate 22: TS of gizzard of *E. nicholsoni* (2.5×10)

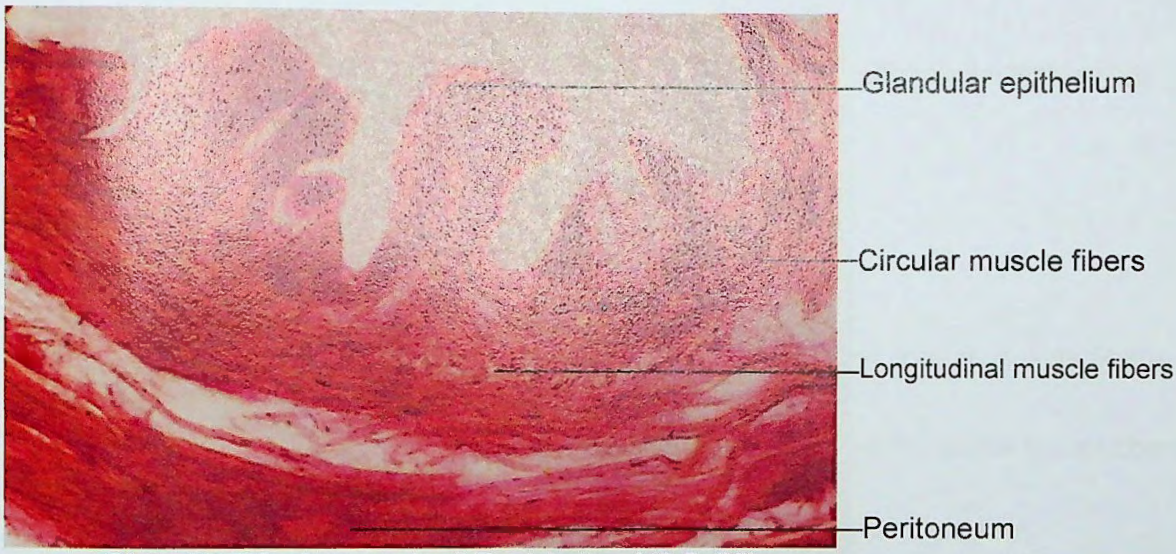


Plate 23: TS of stomach of *E. nicholsoni* (2.5×20)

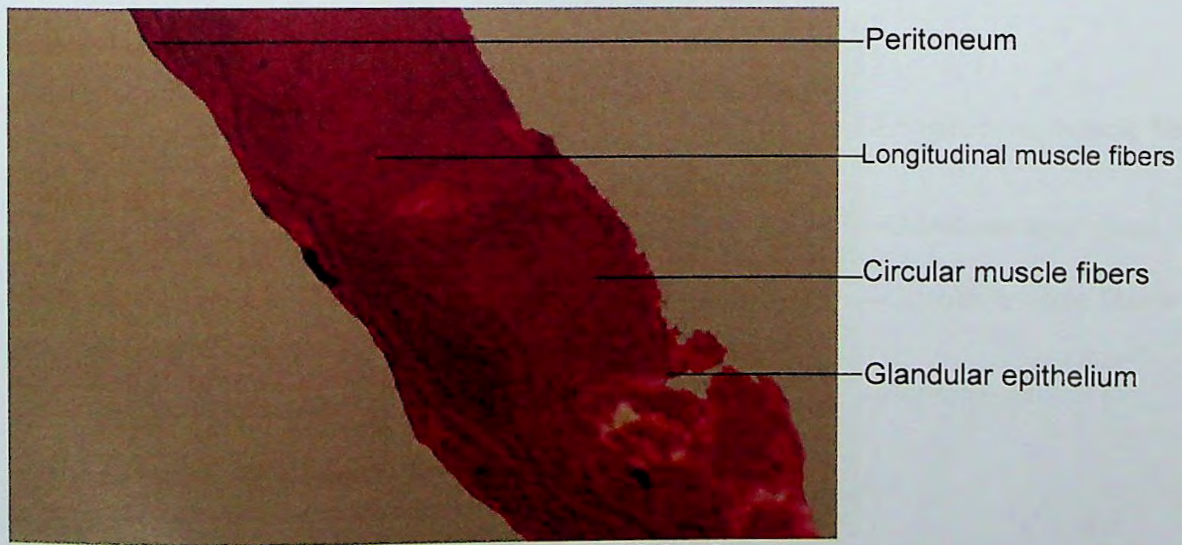


Plate 24: TS of intestine of *E. nicholsoni* (2.5×40)

Plate 25: TS of gizzard of *E. incommodus* (2.5×20)

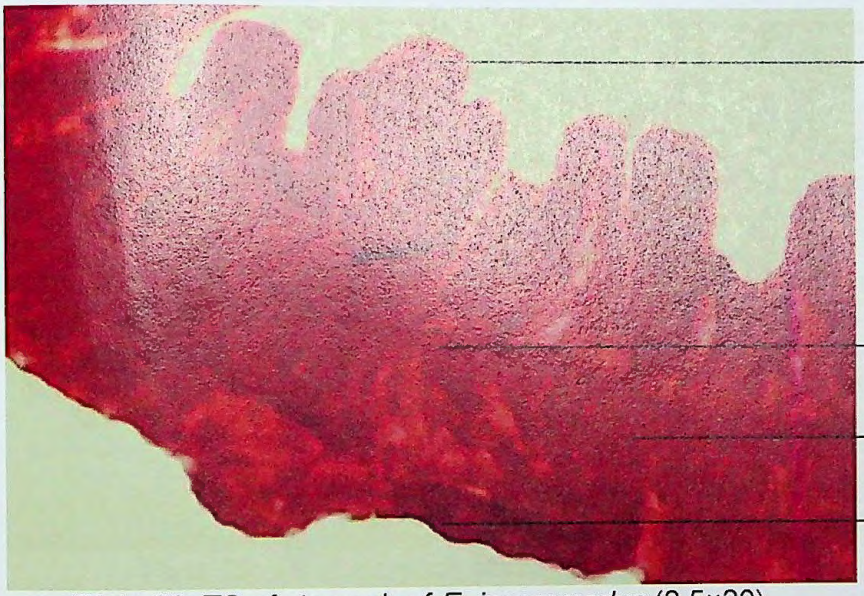


Plate 26: TS of stomach of *E. incommodus* (2.5×20)

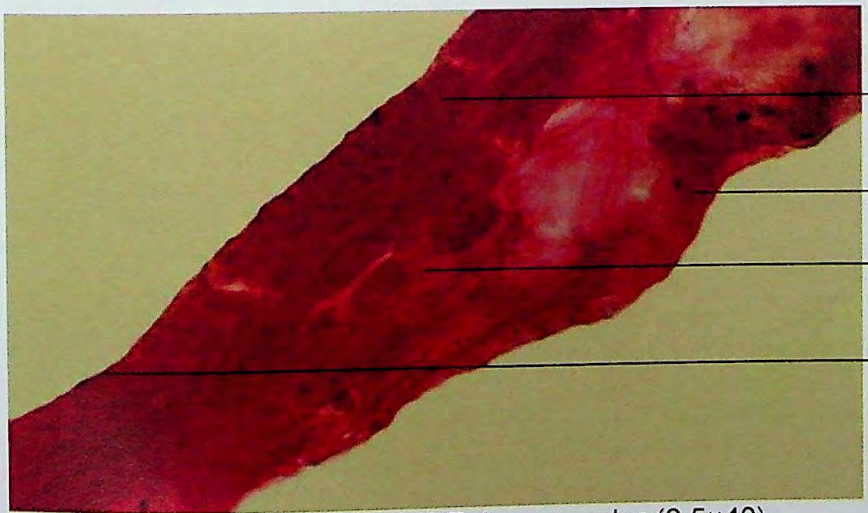


Plate 27: TS of intestine of *E. incommodus* (2.5×40)

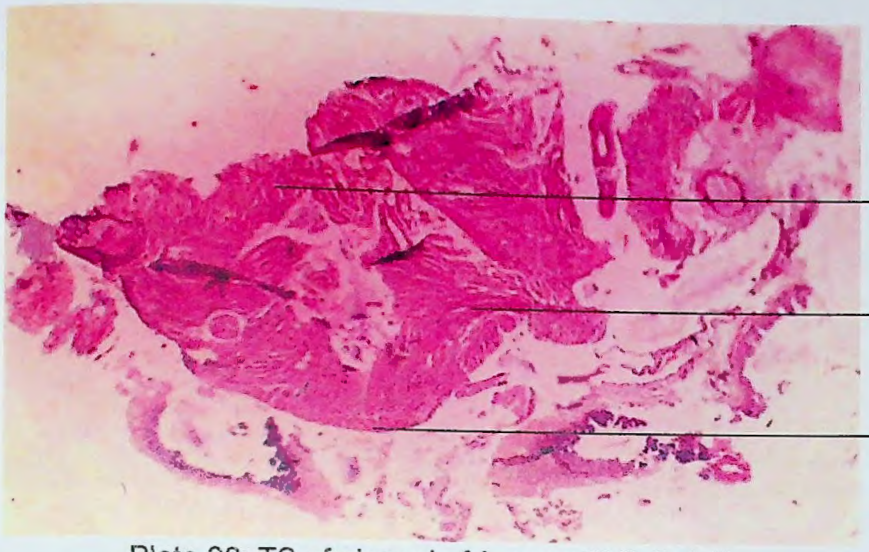


Plate 28: TS of gizzard of *L. mauritii* (2.5×1.5)

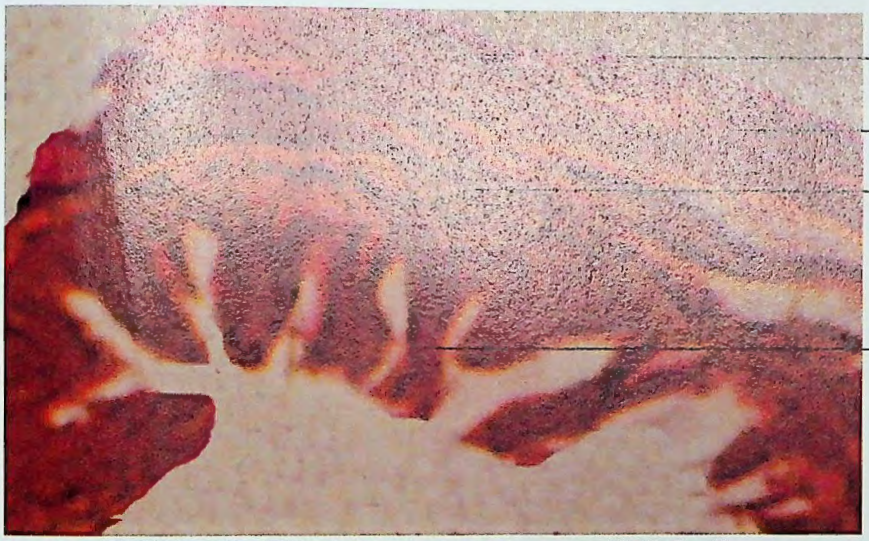


Plate 29: TS of stomach of *L. mauritii* (2.5×4)

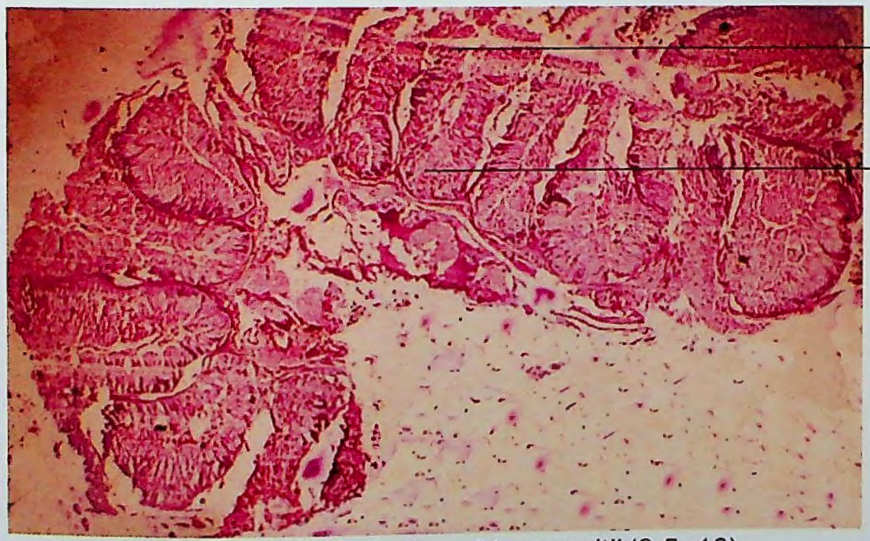


Plate 30: TS of intestine of *L. mauritii* (2.5×10)

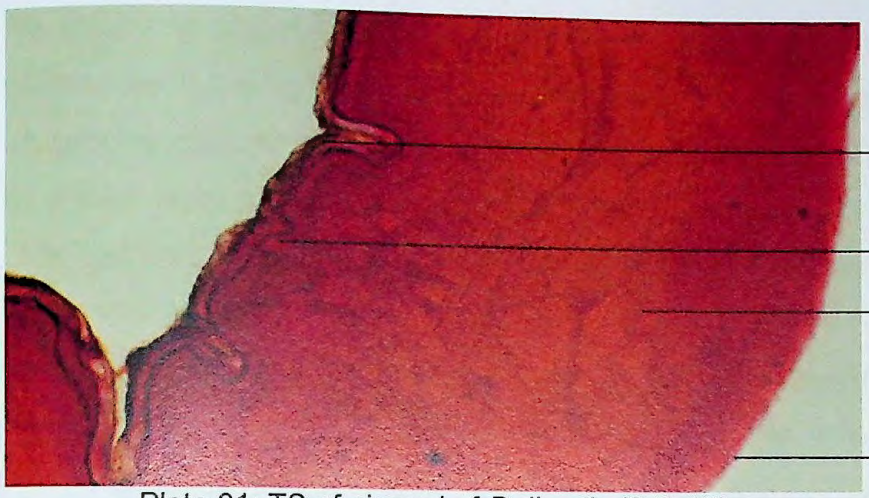


Plate 31: TS of gizzard of *D. limella* (2.5×10)

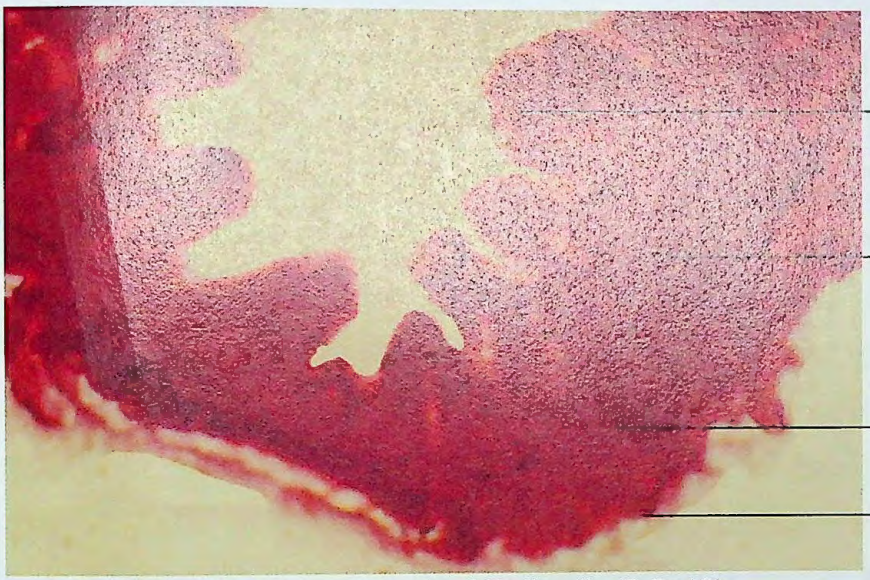


Plate 32: TS of stomach of *D. limella* (2.5×10)

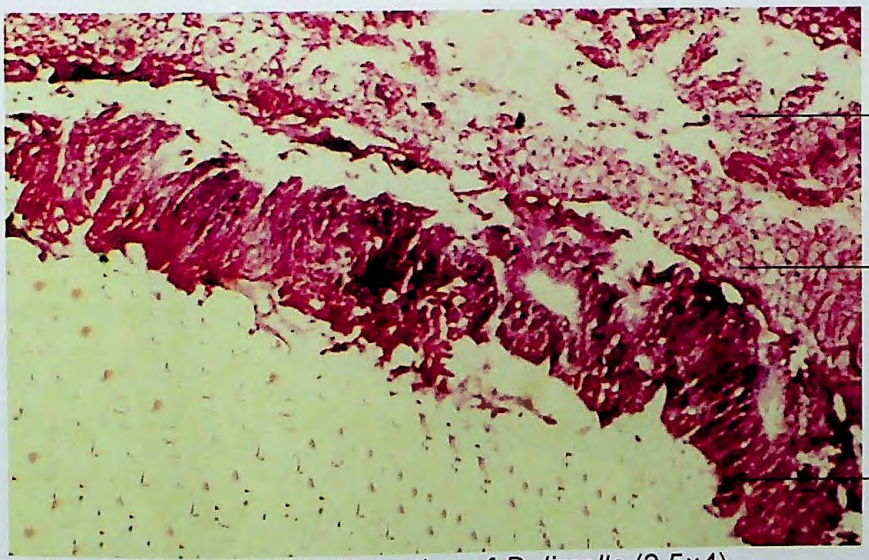


Plate 33: TS of intestine of *D. limella* (2.5×4)

As shown in plates 16, 19, 22, 25, 28 and 31 there are no remarkable differences in the histology of gizzards of six studied species of earthworm. In all species gizzard is composed of an external layer of peritoneum followed by a thick layer of circular muscle fibers, a middle layer of columnar epithelial cells and an internal layer of tough cuticle which is secreted by the underlying columnar epithelial cells. In gizzard circular muscles are unstriped or involuntary and much more developed than that in stomach and intestine. Longitudinal muscles are totally absent in gizzard. However, in *M. posthuma* and *L. mauritii* cuticular layer was thinner than that of other species. The thickest cuticular layer is found in gizzard of *E. incommodus*. In *E. orientalis*, *E. nicholsoni* and *D. limella* epithelial and cuticular layer of gizzard are equally thick and prominent but these two layers is less folded in *E. nicholsoni* than remaining two species.

Stomach of the six studied earthworm species (Plate 17, 20, 23, 26, 29 and 32) consist of outer layer of peritoneum followed by longitudinal muscle fibers, middle layer of circular muscle fibers and inner layer of glandular epithelial cells. In case of *E. orientalis* longitudinal and circular muscles of stomach is thick and well developed but glandular epithelial layer is ill-developed. Glandular epithelial layer is well developed and internally thrown into folds to form well developed villi in all studied species except *E. orientalis*. Circular muscle layer is thinner and villi are more closely arranged in stomach of *E. incommodus* and *D. limella* than those of *E. nicholsoni* and *L. mauritii*.

As shown in Plate 18, 21, 24, 27, 30 and 33 the outer layer of peritoneum is followed by a layer of longitudinal muscles, middle layer of circular muscles and inner layer of absorptive and glandular epithelial cells in intestine. Glandular layer is internally thrown into folds. Cuticle layer is totally absent. In *E. incommodus* and *E. nicholsoni* intestine are thin and villi are compactly arranged while these are separately arranged in remaining studied species of earthworm.

3.4 Bacterial load of the gut of Earthworm

Data obtained from the study on bacterial load of gut of earthworm from September, 2004 to August, 2005 was presented below in table and graph.

Month	Bacterial load of gut of *Metaphire posthuma* (10^6×cfu/gm)		
	Gizzard	Stomach	Intestine
September	18.50 ± 2.26	61.67 ± 14.72	983.33 ± 147.20
October	9.50 ± 0.84	63.33 ± 8.16	833.33 ± 51.64
November	8.17 ± 0.75	40.00 ± 8.94	416.67 ± 75.28
December	6.83 ± 0.75	36.67 ± 5.16	316.67 ± 75.28
January	5.83 ± 0.98	7.67 ± 1.21	300.00 ± 52.53
February	6.17 ± 0.75	12.50 ± 1.52	350.00 ± 54.77
March	6.67 ± 0.52	15.50 ± 1.05	633.33 ± 103.28
April	7.33 ± 0.82	15.00 ± 1.41	600.00 ± 109.54
May	7.33 ± 0.82	19.33 ± 1.86	683.33 ± 75.28
June	8.33 ± 0.82	35.00 ± 5.48	833.33 ± 81.65
July	8.67 ± 1.03	43.33 ± 8.16	816.67 ± 98.32
August	9.50 ± 1.87	65.00 ± 5.48	866.67 ± 51.64

Table 1: Bacterial load of gut of *Metaphire posthuma*

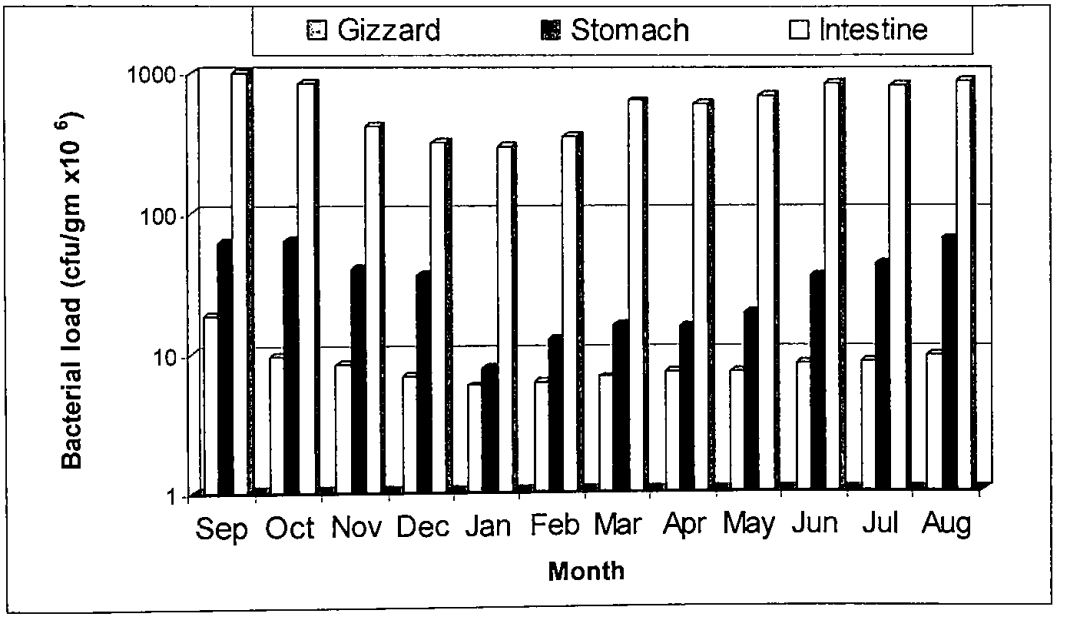


Figure 5: Bacterial load of gut of *Metaphire posthuma*

As result shown in the Table 1 and Figure 5 bacterial load of gizzard, stomach and intestine of *M. posthuma* was the lowest on January. On the other hand, bacterial load of gizzard and intestine was the highest on September, but that of stomach was the highest on August.

Month	Bacterial load of gut of *Eutiphous orientalis* ($\times 10^6$cfu/gm)		
	Gizzard	Stomach	Intestine
September	68.33 ± 17.22	88.33 ± 14.72	1150.00 ± 137.84
October	61.67 ± 7.52	95.00 ± 10.49	1283.33 ± 75.28
November	35.00 ± 5.48	66.67 ± 8.16	800.00 ± 89.44
December	11.33 ± 2.73	56.67 ± 8.16	533.33 ±103.28
January	6.83 ± 0.98	40.00 ± 6.32	270.00 ± 28.28
February	7.83 ± 0.75	50.00 ± 6.32	400.00 ± 89.44
March	8.16 ± 1.17	53.33 ± 8.16	516.67 ± 75.28
April	15.50 ± 2.26	80.00 ± 8.94	733.33 ± 136.62
May	26.83 ± 2.79	76.67± 8.16	783.33 ± 75.28
June	32.50 ± 5.92	98.33 ± 18.34	916.67 ± 147.20
July	41.67 ± 7.53	98.33 ± 13.29	1016.67 ± 194.08
August	63.33 ± 9.16	93.33 ± 10.33	1250.00 ± 137.84

Table 2: Bacterial load of gut of *Eutiphous orientalis*

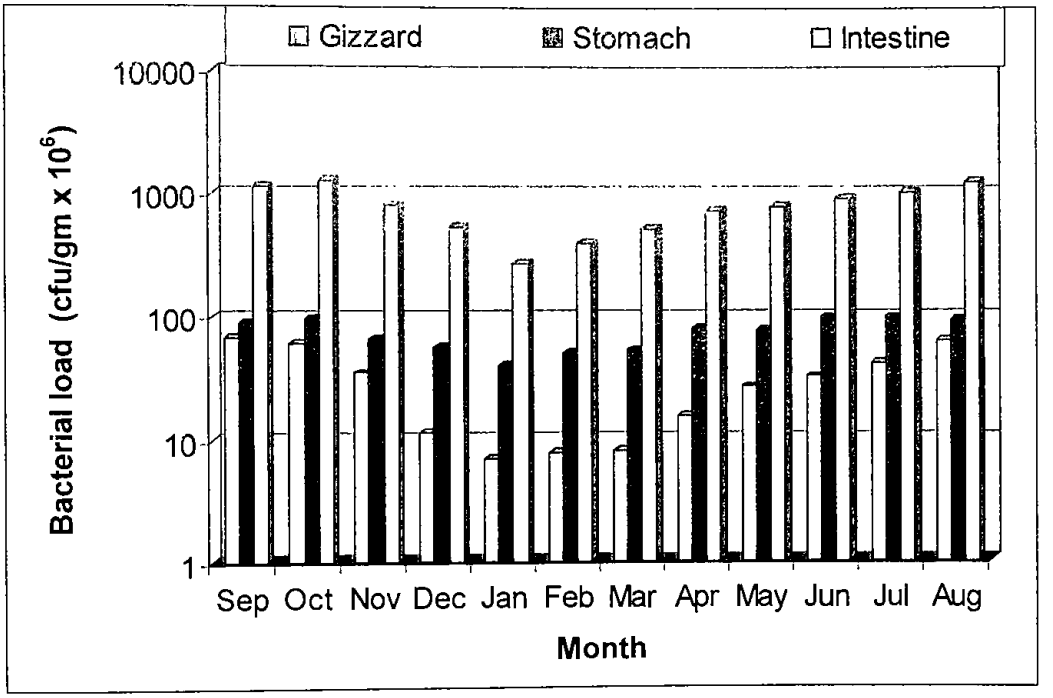


Figure 6: Bacterial load of gut of *Eutiphous orientalis*

The Table 2 and Figure 6 show that the gizzard of *Eutiphous orientalis* contains lower number of bacteria than the other two parts of gut throughout the year. The highest bacterial load of gizzard (68.33 ± 17.22 $\times 10^6$ cfu/gm) was recorded on September. But, the highest bacterial load of stomach (98.33 $\times 10^6$ cfu/gm) was recorded on June and July while that of intestine (1250.00 ± 137.84 $\times 10^6$ cfu/gm) was recorded on August.

Month	Bacterial load of gut of *Eutiphous nicholsoni* (10^6×cfu/gm)		
	Gizzard	Stomach	Intestine
September	78.33 ± 7.53	178.33 ± 11.69	1850.00 ± 104.88
October	85.00 ± 8.37	176.67 ± 12.11	1050.00 ± 104.88
November	48.33 ± 7.53	116.67 ± 12.11	800.00 ± 89.44
December	17.00 ± 1.41	61.67 ± 11.69	550.00 ± 104.88
January	4.67 ± 0.82	13.67 ± 1.03	233.33 ± 8.16
February	6.67 ± 1.21	15.33 ± 1.21	245.00 ± 10.49
March	8.00 ± 0.89	17.67 ± 1.21	285.00 ± 13.78
April	10.67 ± 1.63	48.33 ± 7.53	483.33 ± 75.28
May	13.50 ± 1.38	71.67 ± 7.53	716.67 ± 75.28
June	24.00 ± 1.79	125.00 ± 10.49	1233.33 ± 103.28
July	48.33 ± 7.53	151.67 ± 7.53	1683.33 ± 147.20
August	63.33 ± 8.16	161.67 ± 11.69	1750.00 ± 104.88

Table 3: Bacterial load of gut of *Eutiphous nicholsoni*

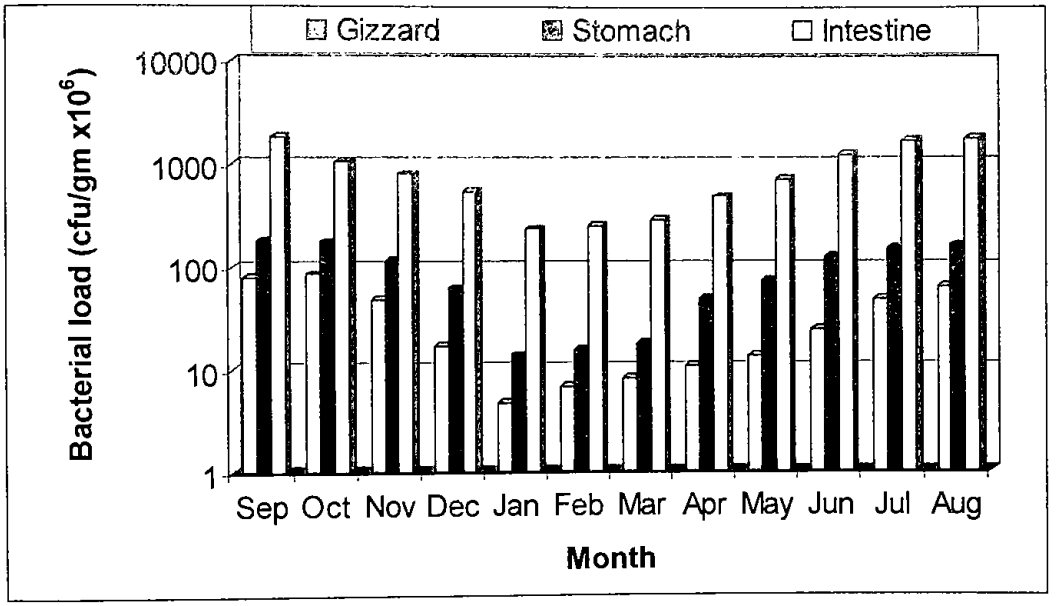


Figure 7: Bacterial load of gut of *Eutiphous nicholsoni*

As in other studied species of earthworm, bacterial load of gizzard of *E. nicholsoni* was lower than that of stomach and intestine. In case of gizzard bacterial load was the highest on October, but in case of stomach and intestine bacterial load was the highest on September.

Month	Bacterial load of gut of *Eutiphous incommodus* (10^6×cfu/gm)		
	Gizzard	Stomach	Intestine
September	85.00 ± 10.49	95.00 ± 17.61	750.00 ± 104.88
October	81.67 ± 9.83	85.00 ± 8.37	178.33 ± 11.69
November	45.00 ± 12.28	43.33 ± 8.16	81.67 ± 7.53
December	12.00 ± 2.97	29.83 ± 5.45	60.00 ± 14.14
January	6.67 ± 0.82	10.00 ± 1.26	78.33 ± 9.83
February	7.33 ± 0.52	14.17 ± 1.17	85.00 ± 5.47
March	7.33 ± 0.82	16.83 ± 1.17	83.33 ± 8.16
April	10.00 ± 1.79	24.50 ± 3.39	105.00 ± 20.74
May	19.00 ± 1.79	29.67 ± 5.39	98.33 ± 11.69
June	27.83 ± 2.32	34.33 ± 6.28	230.00 ± 60.33
July	58.33 ± 9.83	76.67 ± 8.16	346.67 ± 58.88
August	58.33 ± 7.53	83.33 ± 8.16	733.33 ± 121.11

Table 4: Bacterial load of gut of *Eutiphous incommodus*

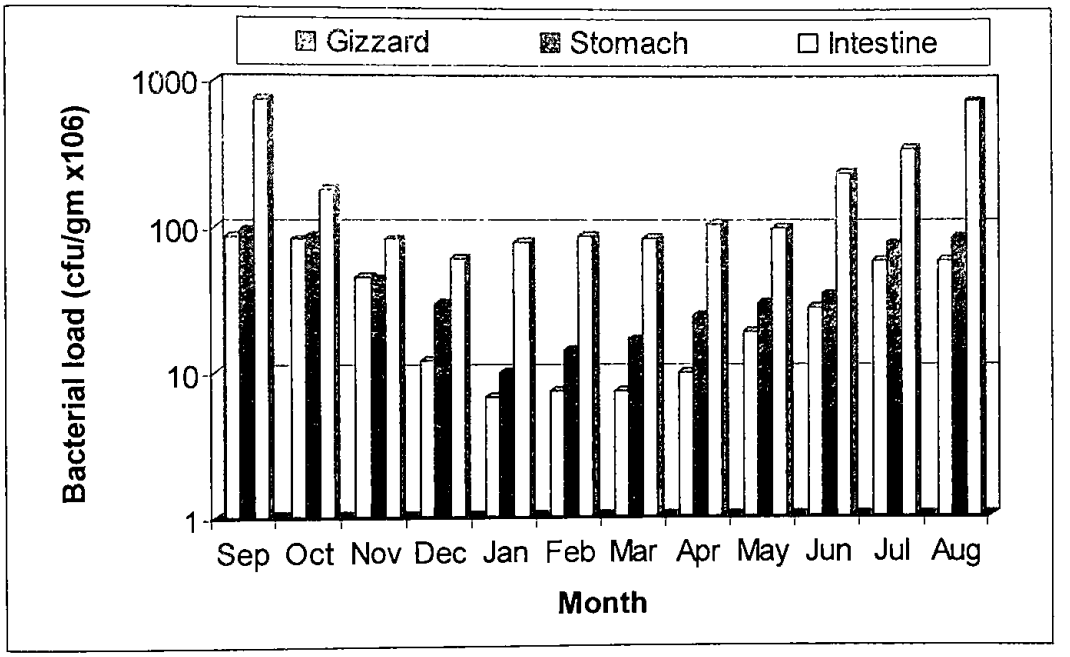


Figure 8: Bacterial load of gut of *Eutiphous incommodus*

Figure 8 show that the bacterial load of gut of *E. incommodus* was the highest on September and the lowest on January. Bacterial load of intestine was higher than that of stomach and gizzard. Highest number of bacteria (750.00 ± 104.88 $\times 10^6$ cfu/gm) was recorded on September in intestine while lowest number (6.67 ± 0.82 $\times 10^6$ cfu/gm) was on January in gizzard.

Month	Bacterial load of gut of *Lampito mauritii* ($\times 10^6$cfu/gm)		
	Gizzard	Stomach	Intestine
September	16.30 ± 1.50	85.00 ± 5.48	833.33 ± 81.65
October	8.50 ± 0.84	65.00 ± 5.48	650.00 ± 104.88
November	7.50 ± 0.84	45.00 ± 5.48	566.67 ± 103.28
December	6.00 ± 0.63	36.66 ± 5.16	516.67 ± 75.28
January	4.83 ± 0.75	13.17 ± 2.64	225.00 ± 47.22
February	6.17 ± 0.75	23.67 ± 3.61	303.33 ± 51.64
March	6.33 ± 0.51	26.33 ± 3.01	466.67 ± 81.65
April	6.50 ± 0.54	28.00 ± 2.53	500.00 ± 109.54
May	6.83 ± 0.75	30.33 ± 5.16	566.67 ± 81.65
June	7.50 ± 0.55	35.00 ± 5.48	616.67 ± 75.28
July	8.17 ± 0.75	46.67 ± 8.16	683.33 ± 98.32
August	9.83 ± 1.83	51.67 ± 7.53	816.67 ± 75.28

Table 5: Bacterial load of gut of *Lampito mauritii*

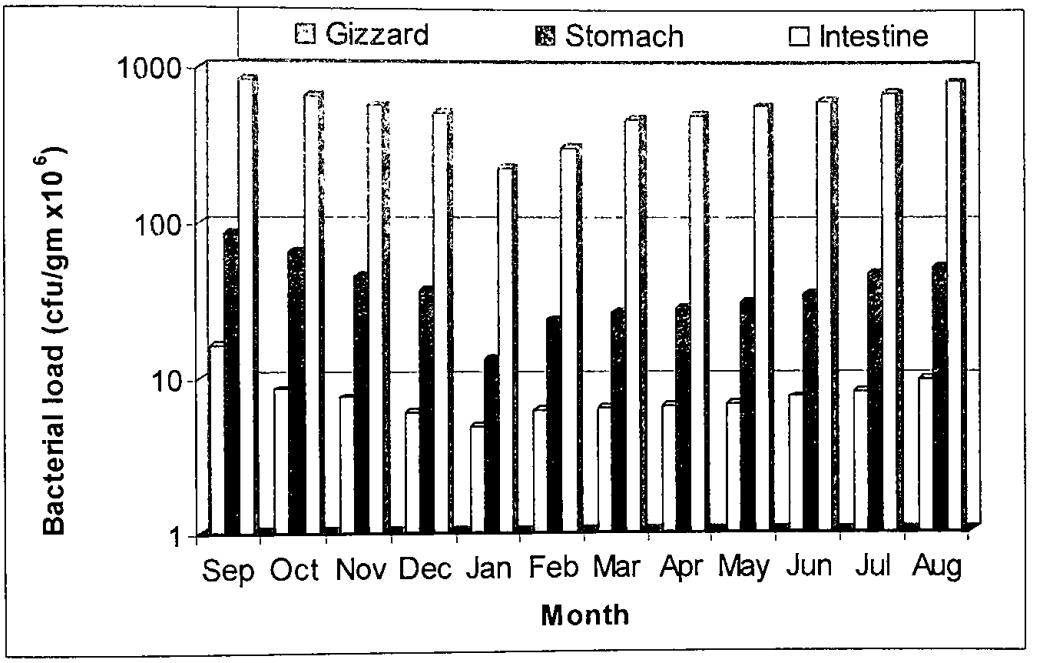


Figure 9: Bacterial load of gut of *Lampito mauritii*

Data shown in the Table 5 and Figure 9 indicate that the bacterial load vary with moth and different parts of gut i.e. gizzard, stomach and intestine. Bacterial load of intestine was higher than that of stomach and gizzard in *L. mauritii* throughout the whole year. The lowest bacterial load (4.83 ± 0.75 $\times 10^6$ cfu/gm) was recorded in gizzard on January while the highest bacterial load (833.33 ± 81.65 $\times 10^6$ cfu/gm) was recorded in intestine on September. Bacterial load of stomach was in between the gizzard and intestine.

Month	Bacterial load of gut of *Drawida limella* (×10^{6}cfu/gm)		
	Gizzard	Stomach	Intestine
September	95.00 ± 17.61	98.33 ± 18.35	1033.33 ± 196.64
October	81.67 ± 7.53	93.33 ± 17.51	800.00 ± 89.44
November	50.00 ± 8.94	56.67 ± 8.16	833.33 ± 81.65
December	9.83 ± 2.14	11.17 ± 1.94	245.00 ± 10.49
January	5.17 ± 0.75	7.00 ± 0.89	65.00 ± 5.48
February	7.33 ± 0.82	8.17 ± 0.75	78.33 ± 7.53
March	8.17 ± 0.75	10.17 ± 1.72	165.00 ± 25.88
April	8.67 ± 0.52	10.83 ± 2.23	225.00 ± 18.71
May	20.67 ± 2.58	20.17 ± 2.14	245.00 ± 18.71
June	25.17 ± 3.87	28.00 ± 3.16	250.00 ± 17.89
July	30.50 ± 4.97	36.67 ± 5.16	416.67 ± 75.28
August	63.33 ± 8.16	71.67 ± 7.53	683.33 ± 75.28

Table 6: Bacterial load of gut of *Drawida limella*

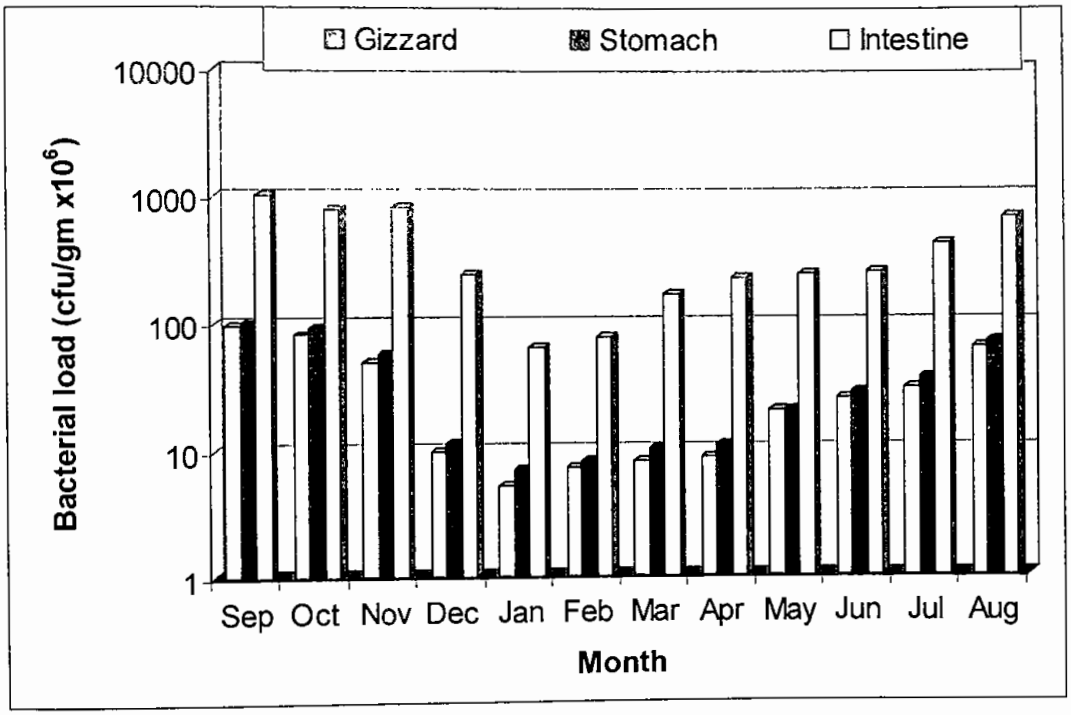


Figure10: Bacterial load of gut of *Drawida limella*

In case of *D. limella* bacterial load of gizzard was lower than that of stomach and intestine throughout the year except on May (Table 6). On May bacterial load of gizzard (20.67 ± 2.58 ×10^6 cfu/gm) was higher than that of stomach (20.17 ± 2.14 ×10^6 cfu/gm). The highest number of bacteria (1033.33 ± 196.64 ×10^6 cfu/gm) is found in intestine on September while lowest number of bacteria (5.17 ± 0.75 ×10^6 cfu/gm) is found in gizzard on January.

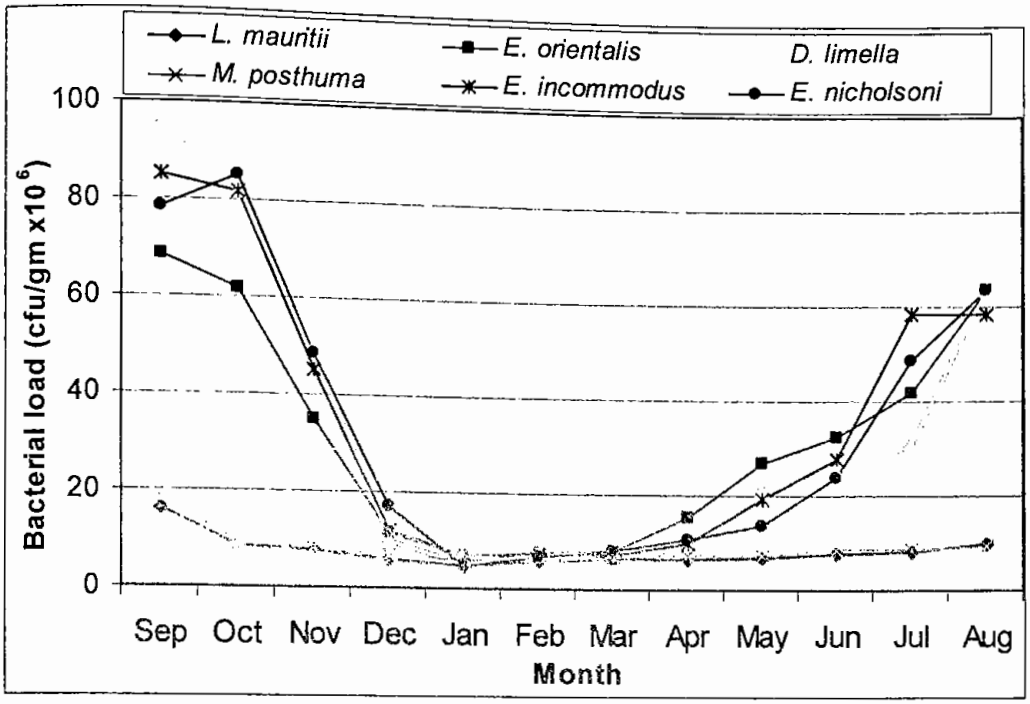


Figure 11: Bacterial load of gizzard of studied earthworm species.

The result shown in figure 11 indicates that bacterial load of gizzard vary with both month and species. Bacterial load of gizzard of all studied species was more or less same from January to March. Remarkable monthly variation of bacterial load was found in *E. orientalis, E. nicholsoni, E. incommodus* and *D. limella* from April to December. But, in case of *M. posthuma* and *L. mauritii* remarkable monthly variation of bacterial load was found only on September. The bacterial load of gizzard of all species tended to increase up to September except *E. nicholsoni* in which bacterial load of gizzard increased up to October. In all species, from September or October bacterial load of gizzard tended to decrease up to December. The highest bacterial load of gizzard ($95.00 \pm 17.61 \times 10^6$ cfu/gm) was recorded in *D. limella* on September while the lowest load ($4.67 \pm 0.82 \times 10^6$ cfu/gm) was recorded in *E. nicholsoni* on January.

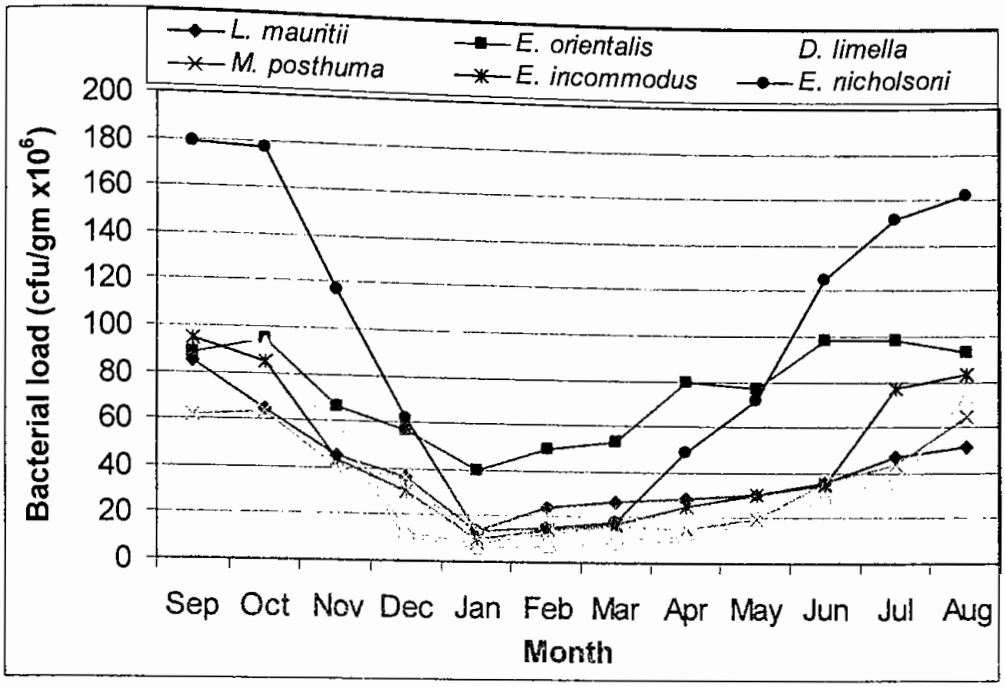


Figure 12: Bacterial load of stomach of studied earthworm species

Figure 12 show that bacterial load of stomach also vary with both month and species. Among the studied species monthly variation of bacterial load of stomach was the most prominent in *E. nicholsoni.* It was the least prominent in *M. posthuma.* In all species the lowest bacterial load was recorded on January. But, the highest bacterial load was recorded on September or October in all species except *E. orientalis.* In case of *E. orientalis* the highest bacterial load was recorded on June and July. Bacterial load of stomach of *E. orientalis* was higher than that of the other species from January to May, but that of *E. nicholsoni* was higher than that of the other species from June to December. Bacterial load of stomach of *D. limella* was lower than that of the other species from December to July. But, miscellaneous result was found on lower bacterial load from August to November.

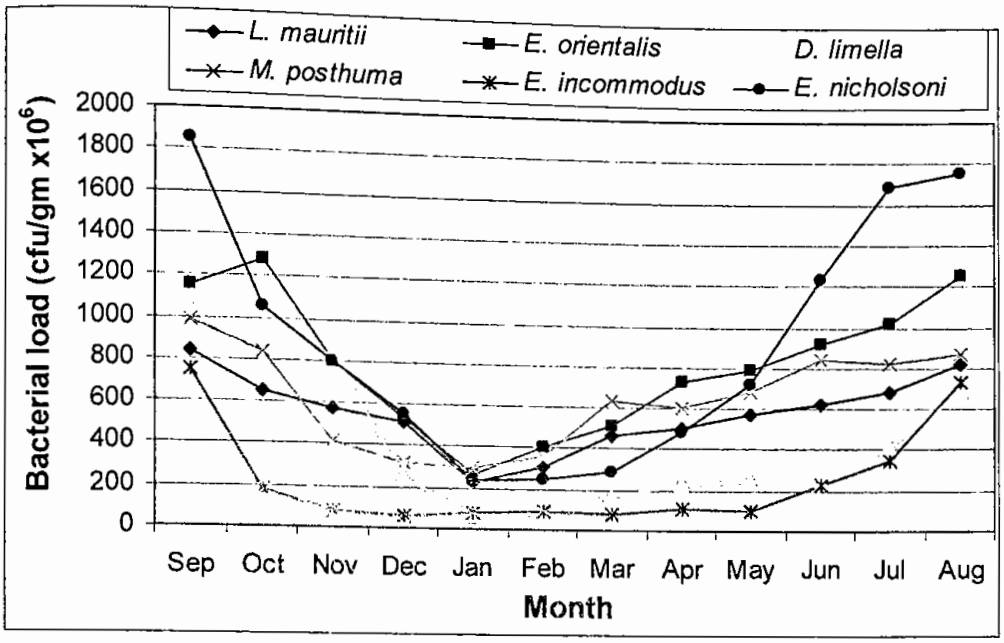


Figure 13 Bacterial load of intestine of studied earthworm species

Monthly variation of bacterial load of intestine was more prominent in *E. nicholsoni* than that of the other species (Figure 13). The least monthly variation of bacterial load of intestine was found in *L. mauritii*. The lowest bacterial load of intestine was recorded on January in all species except *E. incommodus* in which that was recorded on December. The highest bacterial load of intestine (1850.00 ± 104.88×10^6 cfu/gm) was recorded in *E. nicholsoni* on September while the lowest load (60.00 ± 14.14 ×10^6 cfu/gm) was recorded in *E. incommodus* on December.

Data on bacterial load presented in above tables and graphs show that there are variations in bacterial load of gut of different earthworm species, but statistical analysis of the data (F=1.95) indicates that this variation is not significant, that mean bacterial load of gut does not remarkably vary with species of earthworm (Appendix: 29).

Analysis of variance for bacterial load of different parts of gut (F=94.28) indicates that at least bacterial load of one part of gut is significantly different from the others parts of gut (Appendix: 30). Post Hoc Tests for Multiple

Comparisons of bacterial load of different parts of gut make more clear indication that bacterial load of gizzard and stomach is significantly different from that of intestine, but there is no significant difference between bacterial load of gizzard and that of stomach (Appendix: 31).

Analysis of correlation for bacterial load of different parts of gut indicates that there are highly significant correlations among bacterial load of different parts of gut of earthworm (Appendix: 32). It means bacterial load of one part of gut is dependant on that of the other parts. This correlation among bacterial load of different parts of gut is common for all studied species of earthworm (Appendix: 33, 34, 35, 36, 37 and 38).

The data on both monthly variation of bacterial load of gut and factors of weather show that they vary with month. Analysis of correlation between total bacterial load of gut (gizzard, stomach and intestine) and factors of weather indicate that bacterial load of gut is significantly correlated with monthly average rain fall, relative humidity, minimum temperature, soil temperature and sunshine hours (Appendix: 39). There is also a correlation between bacterial load of gut and maximum temperature, but this correlation is statistically insignificant. However, species ways analysis indicates that the correlation between bacterial load of gut and maximum temperature is significant for stomach of *E. orientalis* and intestine of *M. posthuma* (Appendix: 41 and 43). Here, correlation between bacterial load and sunshine hours is negative, but correlation between bacterial load and remaining analyzed factors of weather is positive. It means the increase of rain fall, relative humidity, minimum temperature and soil temperature increases the bacterial load of gut of earthworm. Inversely, the increase of sunshine hours decreases the bacterial load of gut of earthworm.

3.5 Identification of bacteria of earthworm gut

Pure cultures of bacteria collected from gut of different earthworms were identified up to genera. Five genera of bacteria were identified from six species of earthworm. These were *Bacillus, Pseudomonas, Klebsiella, Streptococcus* and *Acinetobacter*. No remarkable variation of bacterial fauna was found in studied species of earthworm. In other ward, these five types of bacteria were found in all studied species of earthworm.

Identified Bacteria	Identifying Characteristics						
	Morphology	Motility	Gram	Catalase	Oxidase	Citrate	Coagulase
Bacillus	Rod	Motile	+	+	+	+	-
Pseudomonas	Rod	Motile	-	+	+	-	-
Klebsiella	Rod	Non-Motile	-	-	-	+	-
Streptococcus	Chained cocci	Non-Motile	+	-	-	+	-
Acinetobacter	Rod	Non-Motile	-	+	-	-	-

Table 7: Identified bacteria and their identifying characteristics

The table 3.7 shows the characteristics of sample bacteria which were used to identify them up to genera. The result shows that *Streptococcus* is chained cocci while *Bacillus, Pseudomonas, Klebsiella* and *Acinetobacter* are rod shaped bacteria. *Bacillus* and *Pseudomonas* are motile bacteria while remaining three are non-motile bacteria. *Bacillus* and *Streptococcus* are gram positive bacteria but *Pseudomonas, Klebsiella* and *Acinetobacter* are gram negative bacteria. Among identified bacteria only *Streptococcus* are catalase negative. *Bacillus* and *Pseudomonas* are oxidase positive while remaining are oxidase negative. On the other hand, *Pseudomonas* and *Acinetobacter* are citrate negative while remaining are citrate positive. All five types of identified bacteria are coagulase negative.

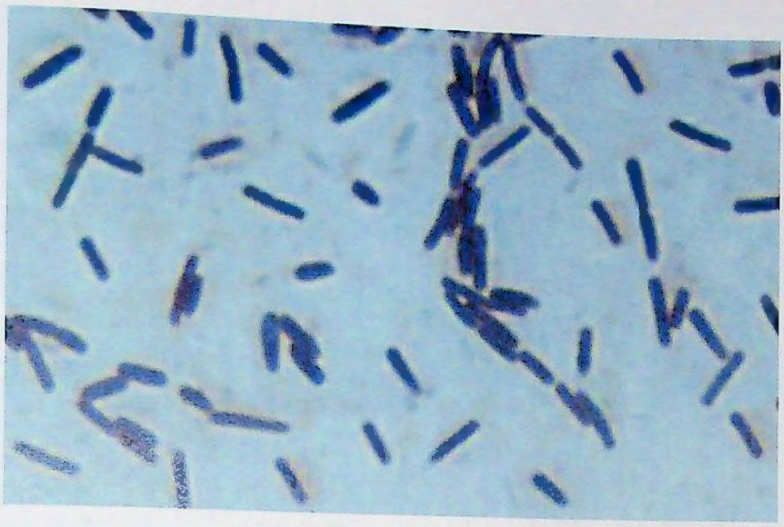


Plate 34: *Bacillus* bacteria

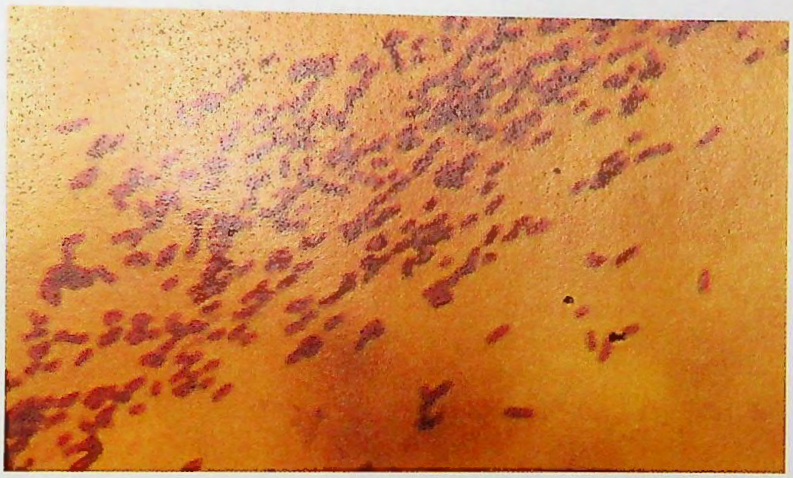


Plate 35: *Pseudomonas* bacteria

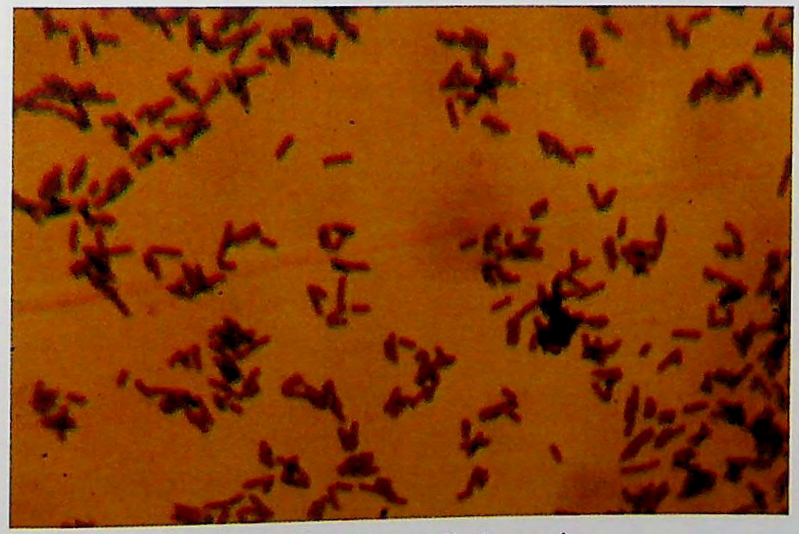


Plate 36: *Klebsiella* bacteria

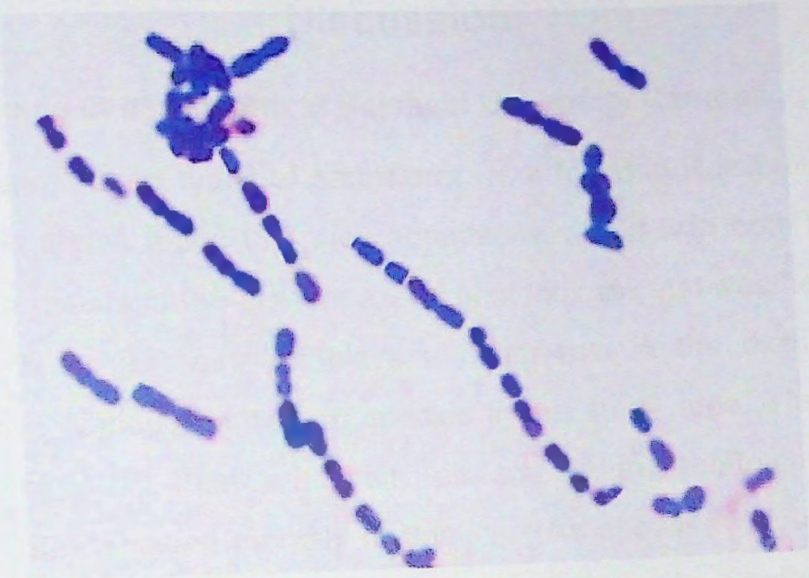


Plate 37: *Streptococcus* bacteria

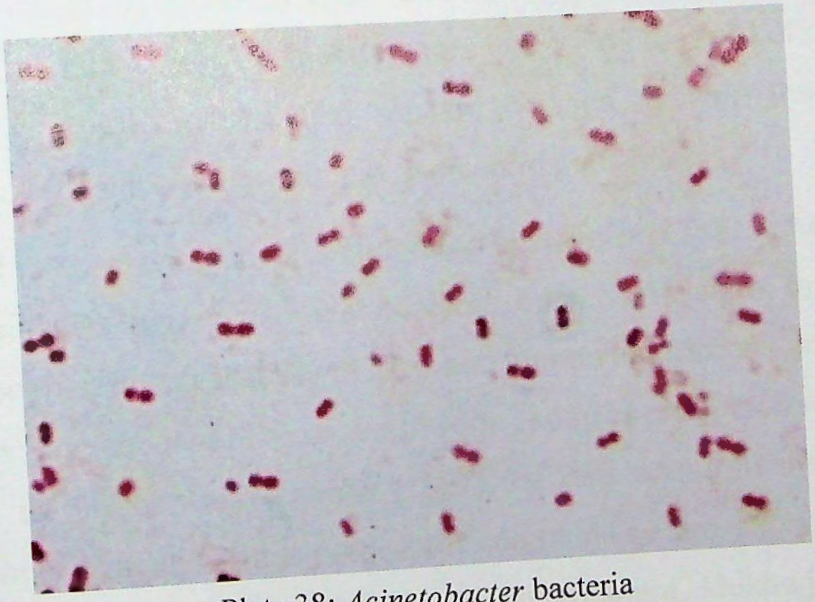


Plate 38: *Acinetobacter* bacteria

Discussion

4.1 Abundance of earthworm at Rajshahi University Campus

In this experiment, six types of earthworm were found in Rajshahi University campus and it was found that their abundance varied with both month and habitat. The results of the present experiment indicate that weather condition coupled with habitat variation plays a major role in the distribution and abundance of various earthworm species in the study area. The factors of weather *i.e.* rain fall, relative humidity, air temperature, soil temperature and sunshine hours showed monthly fluctuation. As shown in Figure 4(a) and 4(b), the rainfall and relative humidity peaked during July- September, which perfectly matched with the maximum abundance of earthworm populations. The soil temperature plays an important role in the maintenance of earthworm population in an ecosystem and present findings indicate the negative correlation of sunshine hours with the earthworm population.

Findings of the present study are supported by several research works. Dash (1998) mentioned that environmental factors, such as moisture, temperature, soil components, pH, availability of food and human interactions influence the distribution and abundance of earthworm species in different habitats. According to Fragoso and Lavelle (1987) the differences in the functional structure and diversity of the earthworm communities are explained by the nutrient richness and heterogeneity of the environment. Mukherjee & Singh (1986) and Edwards & Lofty (1977) reported that earthworm population density and biomass depends not only on the locality and habitat but also on extraction, efficiency, size and age of the worm, edaphic and climatic factors, effects of agricultural practices and chemicals. Reddy and Pasha (1993) reported that the soil types do not seem to influence much on the abundance of earthworms. Edwards and Bohlen (1996) reported that mechanical damage, loss of insulating layer of vegetation, decreased supply of food,

predation by birds could decrease abundance of earthworms in a habitat. They also mentioned that the minimum and maximum population density of earthworms were 5-2020/m^2 as estimated by different authors from different habitats of different countries.

4.2 Morphohistological study of gut of earthworm

In the present experiment, gut of erarthworm is divided into various parts which are mouth, buccal chamber, pharynx, oesophagus, gizzard, stomach, intestine and anus. Kotpal (1995) also functionally divides the gut of earthworm into similar parts. Result of the present study showed that the variation in number, length and position of different parts of guts were common in six studied earthworms. Number and position of gizzard were different in different species of studied earthworms. Similarly, species of studied earthworms varied with each other in length and position of their stomach and intestine. Several authors report the similar findings. Kotpal (1995) stated that in *M. posthuma*, single gizzard is present in the 8^{th} segment, followed by a short and narrow tube like stomach which extends from 9^{th} -14^{th}. Long, wide and thin-walled tube like intestine extends from 15^{th} to the last segment. Intestine is divided into pre-typhlosolar, typhlosolar and post-typhlosolar regions. Stephenson (1923) and Chaudhuri & Bhattacharjee (1999) reported that in *L. mauritii* single distinct gizzard starts from fifth or sixth segment of body, while intestine with rudimentary typhlosole starts from fifteenth segment of body. In *D. limella* two or three gizzards were arranged in 12^{th}-15^{th} or 12^{th}-16^{th} segments and intestine start from 16^{th} or 17^{th} segment (Gates, 1934; Chaudhuri & Bhattacharjee, 1999). Gates (1938) and Stephenson (1923) reported that in *E. incommodus* single large gizzard is in the gap between 6^{th} and 8^{th} segment. Intestine without lateral caecum starts from 13^{th} or 14^{th} segment and extend up to end of the body. They also reported that in *E. nicholsoni* large single gizzard is in 7^{th} segment and intestine without lateral caecum starts from 15^{th} segment. Similarly, in *E.*

orientalis large single gizzard is in 7th segment but intestine without lateral caecum starts from 15th or 16th segment (Gates, 1938., Stephenson, 1923).

Result of present study indicates that there was no remarkable difference in the histology of gut of six studied species of earthworm. In all species gizzard, stomach and intestine are composed of more or less similar layer of muscles. However, few differences were found in thickness and folding of the muscle layer gizzard, stomach and intestine. Literatures on comparative histological study of gut of earthworm are not available. Kotpal (1995) stated the histology of *M. posthuma* which is similar to the findings of present study. He stated that gut is composed of peritoneum, muscle, enteric epithelium and cuticle. Cuticle layer is thick in gizzard. Wall of stomach is highly vascular and glandular and thrown into transverse folds. Internal lining of intestine is ciliated, folded, vascular and glandular. Royuela *et al.*(2005) were studied muscles in intestinal wall of an oligochaete annelid (*Eisenia foetida*) by electron microscopy. The muscle cells in gut are variants of obliquely striated muscle cells. The cells forming the intestinal wall are characterized by their large thick myofilaments (50-52 nm centrally and 27-28 nm at the tips) and abundance of mitochondria. Millott (1944) reported that the intestinal epithelium of *Lumbricus* is composed of ciliated and glandular cells, each of the latter being surrounded by a sheath composed commonly of four or five of the former. It is subject to phasic change, its characters, especially those of the free border, undergoing alteration under varying physiological conditions. In certain of these phases, the free tips of the gland cells of gut of *Lumbricus* are completely over-arched by the free ends of the ciliated cells, which thus form a continuous cover over them. This was noted by Greenwood (1892) who, in addition, observed that at certain phases the covering border was penetrated by inconspicuous openings. It is natural to suspect that the liberation of secretion is correlated with changes in the free ends of the ciliated cells, and may therefore take place during certain of these phases only. Both Greenwood (1892) and Gurwitsch (1901) hint strongly at the idea. Millott (1948) stated that the most remarkable features of the epithelium of gut

orientalis large single gizzard is in 7th segment but intestine without lateral caecum starts from 15th or 16th segment (Gates, 1938., Stephenson, 1923).

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of *Lumricus* are both associated with the ciliated cells, viz. the presence of a complex fibrillar apparatus and associated pore-rings, and the variable appearance of the free border. Concerning the former similar fibrils in epithelial and especially ciliated cells have been described in widely different forms by many workers (Rio-Hortega, 1917). Butschli (1894) denied the existence of fibrilar structures in general, believing they were formed of the mesh work between lines of alveoli, Saguchi (1917), extending this conception, believed that the fibrils were formed by the deeply staining, longitudinal shafts of a cytoplasmic reticulum, Leydig (1883), regarded them as folds of the cell membrane.

4.3 Bacterial load of the gut of Earthworm

It was found in the present experiment that the bacterial load of the gut of earthworm varied with parts of the gut, months and species of earthworm. In all studied earthworms the lowest number of bacteria was recorded in gizzard and followed by stomach. The highest number of bacteria was recorded in intestine. It indicates that the load of bacteria in gut gradually increases from gizzard toward intestine. Variation of bacterial load of one part of gut was significantly correlated with that of the other part of gut. Variation of bacterial load of gut was also significantly correlated with the variation of different factors of weather. These variations of bacterial load in different parts of gut of earthworm could be supported by several studies (Edwards & Bohlen, 1996., Ihssen *et al.,* 2003., Karsten & Drake, 1995, 1997). Several studies have found earthworm-induced increases in microbial biomass (Bohlen *et al.,* 1999). Earthworms can consume large quantities of organic soil matter, surface litter, and mineral soil, and pass organic and inorganic matter through their guts, which subsequently stimulates microbial activities (Edwards & Bohlen, 1996). Sharma *et al.* (2005) reported that during progress through digestive system there is a dramatic increase in number of micro organisms of upto 1000 times. Kale (1991) stated that the total

microbial load in the different regions of the gut of worms has also shown more intense colonization of microbes in the anterior part of the intestine than the other region. According to Byzov *et al.,* (1996) the ingested bacteria partially survive the midgut passage and then multiply with a high growth rate in the hindgut of the animals. Activation of dormant soil bacteria during passage through the gastrointestinal tract of the earthworm could theoretically account for the high numbers of cultured microorganisms detected in gut contents of the earthworm (Ihssen *et al.,* 2003., Karsten & Drake, 1995, 1997). Similarly, Drake & Horn (2007) reported that the anoxic conditions and availability of high quality organic carbon in the earthworm gut theoretically favor microorganisms capable of anaerobic growth. They also stated that despite the deficiency of molecular oxygen in the gut, the viable counts of microbes capable of aerobic growth are also higher in the gut than in pre-ingested soil. Endospores from bacilli and clostridia are common in soil (Garbeva *et al.,* 2003., Ovreas & Torsvik, 1998), and their germination is induced by amino acids and sugars (Setlow, 2003), both of which are abundant in the earthworm gut. Indeed, endospores appear to germinate during gut passage resulting in higher number of bacillus bacteria in gut content (Fischer *et al.,* 1997). Digestion of large ingested bacteria may also occur during gut passage (Brown & Doube, 2004., Clegg, *et al.,* 1995), although total and culture-dependent bacterial counts tend to increase (Fischer *et al.* 1994., Parle, 1963., Pedersen & Hendriksen, 1993., Wolter and Scheu, 1999). Such findings support the conclusion that the number of cultured ingested aerobes increases during passage through gut of earthworm.

Monthly variation of bacterial load of gut of earthworm was found in the present experiment. This monthly variation of bacterial load was correlated to the variation of components of weather. Similar seasonal variation has been documented in microbial communities in numerous terrestrial and aquatic environments (Kevin *et al.,* 2004). In a season enormous numbers of microbial species become inactive and waiting for favorable conditions to

initiate their growth to be accumulated in soil (Torsvik *et al.,* 1990). Murata *et al.* (1999) reported that soil microbial biomass N content showed seasonal variation, gradually increasing from April to August, and decreasing rapidly by the end of August when soil moisture content decreased. Yang *et al.* (2006) stated that summer season had higher microbial populations, biomass and organic content than winter season. Seasonal changes in soil moisture, soil temperature and C input have a large effect on soil microbial biomass and its activity (Ross, 1987). Microbial biomass has been reported to vary seasonally in European soils (Patra *et al.,* 1990). Singh *et al.* (1989) have also reported a seasonal variation in the microbial C, N and P in forests and savanna. Short-term fluctuations of moisture and temperature conditions have been shown to influence the amount of microbial biomass carbon (MacGill *et al.*, 1986). Brown (1995) reported that load of gut microflora are depended on soil environment and food ingested. Microbial composition of ingested soil is the major factor affecting the further population changes during the passage through the earthworm gut (Brown and Doube, 2004). Thus, seasonal variation of bacterial load of ingested soil could be a cause of seasonal variation of bacterial load of earthworm gut.

Idowu *et al.* (2006) also reported that habitats of earthworm are one of the factors that determine the bacterial load of gut of an earthworm. They found that the refuse dump area had the highest numbers of both aerobic and anaerobic organisms, followed by the arboretum while the cultivated land area recorded the lowest counts.

Brown (1995) reported that gut microflora are also preferentially stimulated or reduced depending on earthworm and microbe species. In the present experiment, the bacterial load of gut varies with earthworm species, but this variation was statistically insignificant. In indicate that both internal and external factors are important for bacterial load of gut of earthworm. Antimicrobial activity of coelomic fluid of earthworm varies species to species (Cooper *et al.,* 1969). This variation may influence bacterial load of gut of different earthworm species. Cooper *et al.* (1969) reported that coelomic fluid

of the earthworm, *L. terrestris* had no antibacterial activity when tested against a group of seven different bacterial strains. However, coelomic fluid of *Eisenia foetida* contains naturally occurring factors that are both hemolytic and antibacterial (Roch *et al.,* 1981). Therefore, antimicrobial activity of coelomic fluid of earthworm is still a debating issue.

4.4 Identification of bacteria of earthworm gut

In the present five genera of bacteria were identified from six species of earthworm. The identified bacteria i.e. *Bacillus, Pseudomonas, Klebsiella, Streptococcus* and *Acinetobacter* were common in all studied earthworm. Similar bacteria in gut of earthworms are reported by several authors (Idowu *et al.*, 2006., Ihssen *et al.*, 2003., Fisher *et al.,* 2003). All types of bacteria not grow on nutrient agar media aerobically which was used in present experiment. As a result, beside these five genera other types of bacteria also reported by many authors. Jolly *et al.* (1993) reported that the majority of organisms in gut of earthworm were coccoid, some were filamentous, and a few rod-shaped cells. Bacteria isolated from gut of earthworm were identified as *Staphylococcus, Bacillus* spp., *Pseudomonas aeruginosa, Streptococcus mutans, Clostridium, Spirocheata spp., Azotobacter* spp., *Micrococcus lylae, Acinetobacter* spp., *Halobacterium* (Idowu *et al.*, 2006). Ihssen *et al.* (2003) reported several species of *Bacilli, Paenibacillus* and *Clostridium* in gut of the earthworm, *Aporrectodea caliginosa*. Kim *et al.* (2004) reported twelve groups of bacteria belonging to genera *Aeromonas*, *Agromyces*, *Bacillus*, *Bosea*, *Gordonia*, *Klebsiella*, *Microbacterium*, *Nocardia*, *Pseudomonas*, *Rhodococcus*, *Tsukamurella*, and *Streptomyces* in gut of earthworms. Earthworms might become a vector for mycobacteria (Fisher *et al.,* 2003). Reyes (1976), Mariaglieti (1979) and Contreras (1980) reported that *Vibrio* sp., *Aeromonas hydrophila* and *Streptomyces lipmanii* are common bacteria of the gut of earthworms *Eisenia lucens* and *Pheretima sp*.

The finding of the present experiment that the identified five types of bacteria were common in all species of studied earthworms is also supported by several studies. Drake *et al.* (2006) and Karsten and Drake (1995) stated that the major difference between fermentative microbes cultured from gut contents and preingested soil is quantitative, not qualitative. Thus, the types of bacteria found in the gut appear to be qualitatively representative of the types of bacteria found in soil. Egert *et al.* (2004) reported that there are no dominant indigenous microbes in the gut of *L. terrestris.* Davidson and Stahl (2006) stated that indigenous microbes are present only in excretory organ, the nephridia of earthworm. The lumbricid earthworms harbor gram-negative bacteria in their excretory organs, the nephridia. Comparative 16S rRNA gene sequencing of bacteria associated with the nephridia of several earthworm species has shown that each species of worm harbors a distinct bacterial species (Davidson and Stahl, 2006). Schramm *et al.* (2003) also reported that earthworm-specific microbial symbiont is the occurrence of *Acidovorax* species in earthworm nephridia. But, evidence to date for a quantitatively significant gut-specific microbiota is scant (Jolly *et al.*, 1993; Mendez *et al.*, 2003).

Summary

1. Six species of earthworm identified from three different habitats i.e. shady land, cropland and beside the drainage of residential hall at Rajshahi University Campus. The identified species are *Metaphire posthuma*, *Eutyphoeus orientalis, Eutyphoeus nicholsoni, Eutyphoeus incommodus, Lampito mauritii* and *Drawida limella.*

2. The abundance and distribution of earthworm varied with month and habitats. Weather condition coupled with habitat variation plays a major role in the distribution and abundance of various earthworm species in the study area. But, monthly variation of abundance of earthworm is much more significant than the variation of abundance for different habitats. The rainfall and relative humidity peaked during July-September, which perfectly matched with the maximum abundance of earthworm populations. Negative correlation of sunshine hours with the earthworm population was found.

3. The variation in number, length and position of different parts of guts were common in six studied species of earthworms. Number and position of gizzard were different in different species of studied earthworms. Similarly, species of studied earthworms varied with each other in length and position of their stomach and intestine. But, there are no remarkable variations in the histology of their gut. In all species gizzard, stomach and intestine are composed of similar layer of muscles. Few differences were found only in thickness and folding of the muscle layer of gizzard, stomach and intestine.

4. The bacterial load of the gut of earthworm varied with parts of the gut, months and species of earthworm. In all studied earthworms the lowest number of bacteria was recorded in gizzard and followed by stomach. The highest number of bacteria was recorded in intestine. It indicates that the load of bacteria in gut gradually increases from gizzard toward

intestine. Variation of bacterial load of one part of gut was significantly correlated with that of the other part of gut. Variation of bacterial load of gut was also significantly correlated with monthly average rain fall, relative humidity, minimum temperature, soil temperature and sunshine hours. There is also a correlation between bacterial load of gut and maximum temperature, but this correlation is statistically insignificant. Here, correlation between bacterial load and sunshine hours is negative, but correlation between bacterial load and remaining analyzed factors of weather is positive.

5. Five genera of bacteria were identified from six species of earthworm. The identified bacteria were *Bacillus, Pseudomonas, Klebsiella, Streptococcus* and *Acinetobacter.* They were common in all studied earthworm. Uniform types of gut bacteria reveal that there is no species specific bacterial profile for gut of different earthworm.

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APPENDICES

Appendix 1: Composition of saline solution

10% sodium chloride --- ---- ---- = 9.0 cc

10% Aqucous calcium chloride (Anhydrous) ---- = 0.3 cc

Distilled water --- ---- ---- = 90.0 cc

Appendix 2: Composition of used fixatives:

Various kinds of fixative were used for fixation. These fixatives are as follows:

(i) Carnoy's fluid

(ii) Aqueous Bouin's flied and

(iii) Alcoholic Bouin's fluid.

Chemical constituents of the fixatives:

(i) Carnoys fluid:

Absolute alcohol --- --- --- --- = 60 cc

Chloroform --- --- --- --- = 30 cc

Acetic acid (glacial) --- --- --- --- = 10 cc

Chemical constituents of the fixatives:

(ii) Aqueous Bouin's fluids:

Picric acid (Saturated aqueous solution) --- = 75 cc

Formalin (40% HCHO) --- --- --- = 25 cc

Acetic acid (glacial) --- --- --- --- = 5 cc

Aqueous Bouin's fluid showed better result amongst the fixative used. (Fixed for 12-18 hours; transferred to 70% alcohol).

(iii) Alcoholic Bouin's fluid:

Picric acid	= 1 gm
Acetic acid (glacial)	= 15 cc
Formalin (40% HCHO)	= 60 cc
80% Alcohol	= 15 cc

Appendix 3: Composition of the Mayer's albumen solution:

White protein of egg	= 50 ml
Glycerine	= 50 ml
Sodium salysylate	= 1 gm

Appendix 4: Composition of Hexmatoxylin (Delafield's):

Hematoxylin-3-5% in abs. Alcohol	= 100 ml
Amyonia alum-6.25% in aqueous	= 320 ml
Glycivine	= 80 ml

[Deposits for 3 months, filter before use]

Haema crystal	= 4 gm
100% alcohol	= 25 ml
S. S. of ammonia alam	= 400 ml
Glycerine	= 100 ml
Methyl	= 100 ml

Appendix 5: Composition of nutrient agar.

Peptone -	= 5 g/L
Meat extract -	=1 g/L
Yeast extract -	= 2 g/L
Sodium chloride -	= 5 g/L
Agar -	= 15 g/L
pH -	= 7.0 ± 0.2

Appendix 6: Composition of Simmon's Citrate agar.

Ammonium dihydrogen phosphate	---	---	---	= 1.0 g/L
Di-potassium hydrogen phosphate	---	---	---	= 1.0 g/L
Sodium chloride	---	---	---	= 5.0 g/L
Sodium citrate	---	---	---	= 2.0 g/L
Magnesium sulfate	---	---	---	= 0.2 g/L
Bromothymol blue	---	---	---	= 0.08 g/L
Agar-agar	---	---	---	= 13.0 g/L

Appendix 7: Abundance of earthworms in Shady land in different month

Month	Earthworms / 0.048 cubic meter					
	E. nicholsoni	*E. incommodus*	*M. posthuma*	*E. orientalis*	*D. limella*	*L. mauritii*
Sep	4	4	7	5	3	6
Oct	2	3	4	3	1	4
Nov	1	2	2	2	1	2
Dec	0	2	1	1	0	2
Jan	2	1	2	1	1	1
Feb	1	1	2	1	1	2
Mar	1	2	4	2	2	2
Apr	3	3	7	5	1	5
May	2	2	9	6	2	5
Jun	4	3	8	9	2	8
Jul	4	4	6	8	3	10
Aug	6	7	7	11	3	11

Appendix 8: Abundance of earthworm beside drainage of Residential hall area in different months

Month	Earthworms / 0.048 cubic meter			
	E. orientalis	*M. posthuma*	*L. mauritii*	*D. limella*
Sep	7	6	8	5
Oct	5	2	4	2
Nov	4	3	2	1
Dec	3	2	3	1
Jan	3	3	2	2
Feb	2	2	1	1
Mar	2	3	2	2
Apr	4	4	2	3
May	6	6	5	4
Jun	5	5	8	4
Jul	6	5	8	7
Aug	7	10	12	13

Appendix 9: Abundance of earthworm in crop land in different months

Month	Earthworms / 0.048 cubic meter					
	E. nicholsoni	*E. incommodus*	*M. posthuma*	*E. orientalis*	*D. limella*	*L. mauritii*
Sep	5	2	9	8	10	8
Oct	2	2	6	6	7	5
Nov	2	1	3	3	5	3
Dec	3	2	2	2	5	3
Jan	2	1	2	2	4	1
Feb	2	1	3	1	3	2
Mar	3	2	5	3	3	3
Apr	3	1	8	4	2	4
May	2	2	9	6	3	5
Jun	4	3	8	9	5	6
Jul	5	3	7	11	7	9
Aug	7	4	10	12	9	10

Appendix 10: Bacterial load of Gizzard of *Metaphire posthuma* ($\times 10^6$cfu/gm)

Month	Bacterial load of Gizzard of *Metaphire posthuma* ($\times 10^6$cfu/gm)						
	1st sample			2nd sample			Average ± SD
	Rp1	Rp2	Rp3	Rp1	Rp2	Rp3	
September	17	20	17	16	22	19	18.50 ± 2.26
October	11	9	9	10	9	9	9.50 ± 0.84
November	8	9	7	8	8	9	8.17 ± 0.75
December	7	7	6	7	6	8	6.83 ± 0.75
January	6	4	6	6	7	6	5.83 ± 0.98
February	6	5	7	6	6	7	6.17 ± 0.75
March	7	7	6	7	6	7	6.67 ± 0.52
April	8	7	7	8	6	8	7.33 ± 0.82
May	8	7	8	6	8	7	7.33 ± 0.82
June	9	8	9	8	7	9	8.33 ± 0.82
July	9	9	8	7	10	9	8.67 ± 1.03
August	10	9	8	8	13	9	9.50 ± 1.87

Rp= Replication, SD=Standard Deviation

Appendix 11: Bacterial load of Stomach of *Metaphire posthuma* ($\times 10^6$cfu/gm)

Month	Bacterial load of Stomach of *Metaphire posthuma* ($\times 10^6$cfu/gm)						
	1st sample			2nd sample			Average ± SD
	Rp1	Rp2	Rp3	Rp1	Rp2	Rp3	
September	40	50	70	70	60	80	61.67 ± 14.72
October	50	60	70	60	70	70	63.33 ± 8.16
November	40	30	50	50	40	30	40.00 ± 8.94
December	40	40	30	40	30	40	36.67 ± 5.16
January	8	7	9	7	6	9	7.67 ± 1.21
February	11	13	13	15	12	11	12.50 ± 1.52
March	14	16	15	16	17	15	15.50 ± 1.05
April	15	13	16	17	14	15	15.00 ± 1.41
May	17	21	19	22	18	19	19.33 ± 1.86
June	30	40	30	40	30	40	35.00 ± 5.48
July	40	50	30	50	40	50	43.33 ± 8.16
August	60	70	60	70	60	70	65.00 ± 5.48

Rp= Replication, SD=Standard Deviation

Appendix 12: Bacterial load of Intestine of *Metaphire posthuma* (×10^{6}cfu/gm)

Month	Bacterial load of Intestine of *Metaphire posthuma* (×10^{6}cfu/gm)						
	1st sample			2nd sample			Average ± SD
	Rp1	Rp2	Rp3	Rp1	Rp2	Rp3	
September	1000	1200	800	900	1100	900	983.33 ± 147.20
October	800	800	900	800	900	800	833.33 ± 51.64
November	400	300	400	500	400	500	416.67 ± 75.28
December	200	400	300	400	300	300	316.67 ± 75.28
January	250	300	280	300	270	400	300.00 ± 52.53
February	300	400	300	400	400	300	350.00 ± 54.77
March	500	800	600	600	600	700	633.33 ± 103.28
April	400	600	700	700	600	600	600.00 ± 109.54
May	600	700	700	600	800	700	683.33 ± 75.28
June	900	800	900	800	700	900	833.33 ± 81.65
July	800	900	900	700	900	700	816.67 ± 98.32
August	900	800	900	800	900	900	866.67 ± 51.64

Rp= Replication, SD=Standard Deviation

Appendix 13: Bacterial load of Gizzard of *Eutiphous orientalis* (×10^{6}cfu/gm)

Month	Bacterial load of Gizzard of *Eutiphous orientalis* (×10^{6}cfu/gm)						
	1st sample			2nd sample			Average ± SD
	Rp1	Rp2	Rp3	Rp1	Rp2	Rp3	
September	90	70	40	70	60	80	68.33 ± 17.22
October	70	60	70	60	60	50	61.67 ± 7.52
November	30	40	40	30	30	40	35.00 ± 5.48
December	9	12	15	9	14	9	11.33 ± 2.73
January	7	8	7	7	5	7	6.83 ± 0.98
February	8	7	8	8	7	9	7.83 ± 0.75
March	8	9	6	9	8	9	8.16 ± 1.17
April	12	18	15	17	17	14	15.50 ± 2.26
May	23	30	25	27	30	26	26.83 ± 2.79
June	30	40	28	30	40	27	32.50 ± 5.92
July	30	40	50	40	40	50	41.67 ± 7.53
August	60	50	70	70	60	70	63.33 ± 9.16

Rp= Replication, SD=Standard Deviation

Appendix 14: Bacterial load of Stomach of *Eutiphous orientalis* (×10^{6}cfu/gm)

Month	Bacterial load of Stomach of *Eutiphous orientalis* (×10^{6}cfu/gm)						
	1st sample			2nd sample			Average ± SD
	Rp1	Rp2	Rp3	Rp1	Rp2	Rp3	
September	70	80	100	90	80	110	88.33 ± 14.72
October	90	100	90	110	80	100	95.00 ± 10.49
November	70	60	60	80	70	60	66.67 ± 8.16
December	50	60	70	50	60	50	56.67 ± 8.16
January	40	40	50	30	40	40	40.00 ± 6.32
February	50	40	50	50	50	60	50.00 ± 6.32
March	50	40	60	60	60	50	53.33 ± 8.16
April	70	90	70	90	80	80	80.00 ± 8.94
May	70	80	70	70	80	90	76.67± 8.16
June	90	130	80	90	90	110	98.33 ± 18.34
July	90	110	90	80	90	120	98.33 ± 13.29
August	80	110	90	90	90	100	93.33 ± 10.33

Rp= Replication, SD=Standard Deviation

Appendix 15: Bacterial load of Intestine of *Eutiphous orientalis* (×10^{6}cfu/gm)

Month	Bacterial load of Intestine of *Eutiphous orientalis* (×10^{6}cfu/gm)						
	1st sample			2nd sample			Average ± SD
	Rp1	Rp2	Rp3	Rp1	Rp2	Rp3	
September	1300	1200	1000	1100	1300	1000	1150.00 ± 137.84
October	1200	1300	1200	1300	1400	1300	1283.33 ± 75.28
November	900	800	700	800	900	700	800.00 ± 89.44
December	500	500	700	400	600	500	533.33 ±103.28
January	230	300	260	250	280	300	270.00 ± 28.28
February	500	300	500	400	400	300	400.00 ± 89.44
March	500	400	500	600	600	500	516.67 ± 75.28
April	500	800	900	700	700	800	733.33 ± 136.62
May	800	800	900	700	800	700	783.33 ± 75.28
June	900	800	900	900	1200	800	916.67 ± 147.20
July	900	1300	900	800	1000	1200	1016.67 ± 194.08
August	1300	1400	1200	1300	1000	1300	1250.00 ± 137.84

Rp= Replication, SD=Standard Deviation

Appendix 16: Bacterial load of Gizzard of *Eutiphous incommodus* ($\times 10^6$cfu/gm)

Month	Bacterial load of Gizzard of *Eutiphous incommodus* ($\times 10^6$cfu/gm)						
	1st sample			2nd sample			Average ± SD
	Rp1	Rp2	Rp3	Rp1	Rp2	Rp3	
September	90	80	80	70	100	90	85.00 ± 10.49
October	90	70	90	80	90	70	81.67 ± 9.83
November	30	50	50	30	50	60	45.00 ± 12.28
December	9	12	17	13	12	9	12.00 ± 2.97
January	6	8	6	7	6	7	6.67 ± 0.82
February	7	7	8	7	7	8	7.33 ± 0.52
March	8	7	6	7	8	8	7.33 ± 0.82
April	11	9	10	8	9	13	10.00 ± 1.79
May	20	18	19	22	17	18	19.00 ± 1.79
June	27	24	29	30	27	30	27.83 ± 2.32
July	70	50	50	60	70	50	58.33 ± 9.83
August	60	50	60	60	50	70	58.33 ± 7.53

Rp= Replication, SD=Standard Deviation

Appendix 17: Bacterial load of Stomach of *Eutiphous incommodus* ($\times 10^6$cfu/gm)

Month	Bacterial load of Stomach of *Eutiphous incommodus* (cfu/gm)						
	1st sample			2nd sample			Average ± SD
	Rp1	Rp2	Rp3	Rp1	Rp2	Rp3	
September	90	80	90	90	130	90	95.00 ± 17.61
October	80	90	90	90	70	90	85.00 ± 8.37
November	50	50	40	30	50	40	43.33 ± 8.16
December	30	24	27	30	28	40	29.83 ± 5.45
January	9	11	9	10	9	12	10.00 ± 1.26
February	13	16	14	15	13	14	14.17 ± 1.17
March	18	17	16	17	18	15	16.83 ± 1.17
April	25	26	23	30	20	23	24.50 ± 3.39
May	25	30	28	40	29	26	29.67 ± 5.39
June	40	30	27	29	40	40	34.33 ± 6.28
July	80	70	80	70	70	90	76.67 ± 8.16
August	90	70	80	90	80	90	83.33 ± 8.16

Rp= Replication, SD=Standard Deviation

Appendix 18: Bacterial load of Intestine of *Eutiphous incommodus* ($\times 10^6$cfu/gm)

Month	Bacterial load of Intestine of *Eutiphous incommodus* ($\times 10^6$cfu/gm)						
	1st sample			2nd sample			Average ± SD
	Rp1	Rp2	Rp3	Rp1	Rp2	Rp3	
September	900	700	800	800	700	600	750.00 ± 104.88
October	190	170	180	160	180	190	178.33 ± 11.69
November	80	70	90	90	80	80	81.67 ± 7.53
December	60	40	70	80	60	50	60.00 ± 14.14
January	70	90	70	80	70	90	78.33 ± 9.83
February	90	80	90	80	80	90	85.00 ± 5.47
March	80	70	90	90	90	80	83.33 ± 8.16
April	120	100	90	90	90	140	105.00 ± 20.74
May	100	90	90	120	100	90	98.33 ± 11.69
June	200	300	180	300	160	240	230.00 ± 60.33
July	300	400	280	400	300	400	346.67 ± 58.88
August	700	600	800	800	600	900	733.33 ± 121.11

Rp= Replication, SD=Standard Deviation

Appendix 19: Bacterial load of Gizzard of *Eutiphous nicholsoni* ($\times 10^6$cfu/gm)

Month	Bacterial load of Gizzard of *Eutiphous nicholsoni* ($\times 10^6$cfu/gm)						
	1st sample			2nd sample			Average ± SD
	Rp1	Rp2	Rp3	Rp1	Rp2	Rp3	
September	70	80	70	80	90	80	78.33 ± 7.53
October	90	80	90	70	90	90	85.00 ± 8.37
November	40	50	60	40	50	50	48.33 ± 7.53
December	19	16	17	15	17	18	17.00 ± 1.41
January	4	5	4	5	6	4	4.67 ± 0.82
February	7	6	8	6	5	8	6.67 ± 1.21
March	8	7	9	7	9	8	8.00 ± 0.89
April	10	9	12	11	9	13	10.67 ± 1.63
May	12	15	13	14	15	12	13.50 ± 1.38
June	21	24	25	26	23	25	24.00 ± 1.79
July	40	50	40	60	50	50	48.33 ± 7.53
August	60	70	60	70	50	70	63.33 ± 8.16

Rp= Replication, SD=Standard Deviation

Appendix 20: Bacterial load of Stomach of *Eutiphous nicholsoni* (×10^6cfu/gm)

Month	Bacterial load of Stomach of *Eutiphous nicholsoni* (×10^6cfu/gm)						
	1st sample			2nd sample			Average ± SD
	Rp1	Rp2	Rp3	Rp1	Rp2	Rp3	
September	160	180	190	180	190	170	178.33 ± 11.69
October	180	190	170	170	160	190	176.67 ± 12.11
November	100	110	130	120	110	130	116.67 ± 12.11
December	60	60	80	50	70	50	61.67 ± 11.69
January	12	14	14	15	13	14	13.67 ± 1.03
February	14	16	15	17	14	16	15.33 ± 1.21
March	17	17	19	18	19	16	17.67 ± 1.21
April	50	40	40	60	50	50	48.33 ± 7.53
May	70	80	60	80	70	70	71.67 ± 7.53
June	120	140	120	130	130	110	125.00 ± 10.49
July	150	150	140	160	150	160	151.67 ± 7.53
August	150	170	160	160	150	180	161.67 ± 11.69

Rp= Replication, SD=Standard Deviation

Appendix 21: Bacterial load of Intestine of *Eutiphous nicholsoni* (×10^6cfu/gm)

Month	Bacterial load of Intestine of *Eutiphous nicholsoni* (×10^6cfu/gm)						
	1st sample			2nd sample			Average ± SD
	Rp1	Rp2	Rp3	Rp1	Rp2	Rp3	
September	1900	1800	1900	1800	2000	1700	1850.00 ± 104.88
October	1000	1100	1000	1100	900	1200	1050.00 ± 104.88
November	700	800	900	800	900	700	800.00 ± 89.44
December	700	600	500	400	600	500	550.00 ± 104.88
January	220	240	230	240	230	240	233.33 ± 8.16
February	250	240	230	240	250	260	245.00 ± 10.49
March	300	270	290	280	300	270	285.00 ± 13.78
April	500	500	400	600	500	400	483.33 ± 75.28
May	700	800	600	800	700	700	716.67 ± 75.28
June	1200	1100	1200	1300	1200	1400	1233.33 ± 103.28
July	1500	1800	1700	1600	1900	1600	1683.33 ± 147.20
August	1900	1700	1800	1700	1600	1800	1750.00 ± 104.88

Rp= Replication, SD=Standard Deviation

Appendix 22: Bacterial load of Gizzard of *Lampito mauritii* ($\times 10^6$cfu/gm)

Month	Bacterial load of Gizzard of *Lampito mauritii* ($\times 10^6$cfu/gm)						
	1st sample			2nd sample			Average ± SD
	Rp1	Rp2	Rp3	Rp1	Rp2	Rp3	
September	16	15	19	16	17	15	16.30 ± 1.50
October	9	9	8	7	9	9	8.50 ± 0.84
November	7	9	7	7	8	7	7.50 ± 0.84
December	6	7	6	6	6	5	6.00 ± 0.63
January	5	4	4	5	5	6	4.83 ± 0.75
February	6	5	6	7	6	7	6.17 ± 0.75
March	6	7	6	6	6	7	6.33 ± 0.51
April	7	7	6	6	6	7	6.50 ± 0.54
May	8	6	7	6	7	7	6.83 ± 0.75
June	7	8	8	8	7	7	7.50 ± 0.55
July	8	7	9	8	8	9	8.17 ± 0.75
August	11	9	8	9	13	9	9.83 ± 1.83

Rp= Replication, SD=Standard Deviation

Appendix 23: Bacterial load of Stomach of *Lampito mauritii* ($\times 10^6$cfu/gm)

Month	Bacterial load of Stomach of *Lampito mauritii* ($\times 10^6$cfu/gm)						
	1st sample			2nd sample			Average ± SD
	Rp1	Rp2	Rp3	Rp1	Rp2	Rp3	
September	80	90	80	80	90	90	85.00 ± 5.48
October	60	70	70	60	70	60	65.00 ± 5.48
November	40	50	40	50	50	40	45.00 ± 5.48
December	30	40	40	40	40	30	36.66 ± 5.16
January	10	14	11	15	12	17	13. 17 ± 2.64
February	24	30	20	22	25	21	23.67 ± 3.61
March	23	26	30	30	24	25	26.33 ± 3.01
April	30	26	30	30	28	24	28.00 ± 2.53
May	25	30	40	30	30	27	30.33 ± 5.16
June	40	30	40	40	30	30	35.00 ± 5.48
July	40	50	50	60	40	40	46.67 ± 8.16
August	60	50	40	50	50	60	51.67 ± 7.53

Rp= Replication, SD=Standard Deviation

Appendix 24: Bacterial load of Intestine of *Lampito mauritii* ($\times 10^6$cfu/gm)

Month	Bacterial load of Intestine of *Lampito mauritii* ($\times 10^6$cfu/gm)						
	1st sample			2nd sample			Average ± SD
	Rp1	Rp2	Rp3	Rp1	Rp2	Rp3	
September	800	900	700	800	900	900	833.33 ± 81.65
October	700	600	700	500	800	600	650.00 ± 104.88
November	600	600	500	600	700	400	566.67 ± 103.28
December	600	500	600	400	500	500	516.67 ± 75.28
January	200	160	220	250	220	300	225.00 ± 47.22
February	300	300	270	300	250	400	303.33 ± 51.64
March	400	600	400	500	400	500	466.67 ± 81.65
April	400	500	700	500	400	500	500.00 ± 109.54
May	500	700	500	600	600	500	566.67 ± 81.65
June	500	700	600	700	600	600	616.67 ± 75.28
July	600	800	600	700	800	600	683.33 ± 98.32
August	700	900	800	800	800	900	816.67 ± 75.28

Rp= Replication, SD=Standard Deviation

Appendix 25: Bacterial load of Gizzard of *Drawida limella* ($\times 10^6$cfu/gm)

Month	Bacterial load of Gizzard of *Drawida limella* ($\times 10^6$cfu/gm)						
	1st sample			2nd sample			Average ± SD
	Rp1	Rp2	Rp3	Rp1	Rp2	Rp3	
September	90	90	80	130	90	90	95.00 ± 17.61
October	90	80	80	90	70	80	81.67 ± 7.53
November	60	40	50	60	50	40	50.00 ± 8.94
December	12	9	8	9	13	8	9.83 ± 2.14
January	5	6	5	4	5	6	5.17 ± 0.75
February	8	6	8	7	7	8	7.33 ± 0.82
March	9	9	8	7	8	8	8.17 ± 0.75
April	9	9	8	9	8	9	8.67 ± 0.52
May	17	23	21	20	24	19	20.67 ± 2.58
June	23	25	30	26	19	28	25.17 ± 3.87
July	27	30	40	30	30	26	30.50 ± 4.97
August	50	70	70	60	60	70	63.33 ± 8.16

Rp= Replication, SD=Standard Deviation

Appendix 26: Bacterial load of Stomach of *Drawida limella* (×10^{6}cfu/gm)

Month	Bacterial load of Stomach of *Drawida limella* (×10^{6}cfu/gm)						
	1st sample			2nd sample			Average ± SD
	Rp1	Rp2	Rp3	Rp1	Rp2	Rp3	
September	130	90	90	110	80	90	98.33 ± 18.35
October	120	80	90	110	80	80	93.33 ± 17.51
November	50	60	50	70	60	50	56.67 ± 8.16
December	12	14	11	9	12	9	11.17 ± 1.94
January	7	8	7	6	6	8	7.00 ± 0.89
February	8	7	8	9	8	9	8.17 ± 0.75
March	9	12	9	11	8	12	10.17 ± 1.72
April	13	9	11	9	14	9	10.83 ± 2.23
May	19	20	23	17	20	22	20.17 ± 2.14
June	23	30	30	30	25	30	28.00 ± 3.16
July	40	30	40	40	30	40	36.67 ± 5.16
August	70	70	60	80	70	80	71.67 ± 7.53

Rp= Replication, SD=Standard Deviation

Appendix 27: Bacterial load of Intestine of *Drawida limella* (×10^{6}cfu/gm)

Month	Bacterial load of Intestine of *Drawida limella* (×10^{6}cfu/gm)						
	1st sample			2nd sample			Average ± SD
	Rp1	Rp2	Rp3	Rp1	Rp2	Rp3	
September	1300	900	1100	1200	900	800	1033.33 ± 196.64
October	900	800	800	700	900	700	800.00 ± 89.44
November	900	700	900	800	900	800	833.33 ± 81.65
December	230	240	260	240	250	250	245.00 ± 10.49
January	60	60	70	60	70	70	65.00 ± 5.48
February	80	70	80	70	80	90	78.33 ± 7.53
March	190	140	130	180	160	190	165.00 ± 25.88
April	230	250	200	210	240	220	225.00 ± 18.71
May	250	220	260	230	270	240	245.00 ± 18.71
June	240	230	240	280	250	260	250.00 ± 17.89
July	400	300	400	500	400	500	416.67 ± 75.28
August	700	600	600	700	700	800	683.33 ± 75.28

Rp= Replication, SD=Standard Deviation

Appendix 28: Data on weather of Rajshahi city from September, 2004 to August, 2005

Month	Rain fall (Avg.)cm	Relative humidity (Avg.)%	Minimum temperature (Avg.)°C	Maximum temperature (Avg.)°C	Soil temperature (Avg.)°C	Sunshine hour (Avg.)
September	11.6	88.33	25.53	31.47	29.43	3.53
October	5.1	84.33	22.3	31.1	28.06	7.47
November	0	78	16.3	27.8	23.83	9.17
December	0	77.67	14.2	26.4	20.83	7.4
January	0.45	80	11.3	23.8	18	5.76
February	0.03	70	15.06	28.17	21.63	8.4
March	3.46	70.33	19.66	32.9	26.47	7.07
April	0.89	72.33	23.5	35.77	30.33	8.28
May	3.59	76	24.43	35.07	31.16	7.57
June	4	77	26.1	36	32.93	4.96
July	16.44	87.33	26.1	32.3	30.1	3.94
August	5.38	86	26.77	33.13	31.1	4.11

Appendix 29: Analysis of variance for Bacterial load of different earthworm species

Bacterial load	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1729054743008172000.000	5	345810948601634400.000	1.950	.088
Within Groups	37250067864498620000.000	210	177381275545231500.000		
Total	38979122607506790000.000	215			

Appendix 30: Analysis of variance for Bacterial load of different parts of gut

Bacterial load	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	18303339964407200000.000	2	9151669982203600000.000	94.28**	.000
Within Groups	20675782643099600000.000	213	97069402080279800.000		
Total	38979122607506810000.000	215			

** The mean difference is significant at the .01 level.

Appendix 31: Post Hoc Tests for Multiple Comparisons of bacterial load of different parts of gut

Dependent Variable: Bacterial load

	(I) name of part of gut	(J) name of part of gut	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	gizzard	stoamch	-31664351.85	51926604.742	.815	-154222318.79	90893615.08
		intestine	-632733796.29(*)	51926604.742	.000	-755291763.22	-510175829.36
	stoamch	gizzard	31664351.85	51926604.742	.815	-90893615.08	154222318.79
		intestine	-601069444.44(*)	51926604.742	.000	-723627411.37	-478511477.50
	intestine	gizzard	632733796.29(*)	51926604.742	.000	510175829.36	755291763.22
		stoamch	601069444.44(*)	51926604.742	.000	478511477.50	723627411.37
Scheffe	gizzard	stoamch	-31664351.85	51926604.742	.830	-159666613.66	96337909.95
		intestine	-632733796.29(*)	51926604.742	.000	-760736058.10	-504731534.48
	stoamch	gizzard	31664351.85	51926604.742	.830	-96337909.95	159666613.66
		intestine	-601069444.44(*)	51926604.742	.000	-729071706.24	-473067182.63
	intestine	gizzard	632733796.29(*)	51926604.742	.000	504731534.48	760736058.10
		stoamch	601069444.44(*)	51926604.742	.000	473067182.63	729071706.24

* The mean difference is significant at the .05 level.

Appendix 32: Correlations among total bacterial load of gizzard, stomach and intestine of six studied species of earthworm

		Total bacterial load of gizzard of six studied species of earthworm	Total bacterial load of stomach of six studied species of earthworm	Total bacterial load of intestine of six studied species of earthworm
Total bacterial load of gizzard of six studied species of earthworm	Pearson Correlation	1	.984(**)	.941(**)
	Sig. (2-tailed)	.	.000	.000
	N	12	12	12
Total bacterial load of stomach of six studied species of earthworm	Pearson Correlation	.984(**)	1	.964(**)
	Sig. (2-tailed)	.000	.	.000
	N	12	12	12
Total bacterial load of intestine of six studied species of earthworm	Pearson Correlation	.941(**)	.964(**)	1
	Sig. (2-tailed)	.000	.000	.
	N	12	12	12

** Correlation is significant at the 0.01 level (2-tailed).

Appendix 33: Correlations among bacterial load of gizzard, stomach and intestine of *L. mauritii*

		Bacterial load of gizzard of *L. mauritii*	Bacterial load of stomach of *L. mauritii*	Bacterial load of intestine of *L. mauritii*
Bacterial load of gizzard of *L. mauritii*	Pearson Correlation	1	.961(**)	.955(**)
	Sig. (2-tailed)	.	.000	.000
	N	12	12	12
Bacterial load of stomach of *L. mauritii*	Pearson Correlation	.961(**)	1	.981(**)
	Sig. (2-tailed)	.000	.	.000
	N	12	12	12
Bacterial load of intestine of *L. mauritii*	Pearson Correlation	.955(**)	.981(**)	1
	Sig. (2-tailed)	.000	.000	.
	N	12	12	12

** Correlation is significant at the 0.01 level (2-tailed).

Appendix 34: Correlations among bacterial load of gizzard, stomach and intestine of *E. orientalis*

		Bacterial load of gizzard of *E. orientalis*	Bacterial load of stomach of *E. orientalis*	Bacterial load of intestine of *E. orientalis*
Bacterial load of gizzard of *E. orientalis*	Pearson Correlation	1	.791(**)	.951(**)
	Sig. (2-tailed)	.	.002	.000
	N	12	12	12
Bacterial load of stomach of *E. orientalis*	Pearson Correlation	.791(**)	1	.915(**)
	Sig. (2-tailed)	.002	.	.000
	N	12	12	12
Bacterial load of intestine of *E. orientalis*	Pearson Correlation	.951(**)	.915(**)	1
	Sig. (2-tailed)	.000	.000	.
	N	12	12	12

** Correlation is significant at the 0.01 level (2-tailed).

Appendix 35: Correlations among bacterial load of gizzard, stomach and intestine of *D. lamella*

		Bacterial load of gizzard of *D. limella*	Bacterial load of stomach of *D. limella*	Bacterial load of intestine of *D. limella*
Bacterial load of gizzard of *D. limella*	Pearson Correlation	1	.997(**)	.957(**)
	Sig. (2-tailed)	.	.000	.000
	N	12	12	12
Bacterial load of stomach of *D. limella*	Pearson Correlation	.997(**)	1	.955(**)
	Sig. (2-tailed)	.000	.	.000
	N	12	12	12
Bacterial load of intestine of *D. limella*	Pearson Correlation	.957(**)	.955(**)	1
	Sig. (2-tailed)	.000	.000	.
	N	12	12	12

** Correlation is significant at the 0.01 level (2-tailed).

Appendix 36: Correlations among bacterial load of gizzard, stomach and intestine of *M. posthuma*

		Bacterial load of gizzard of *M. posthuma*	Bacterial load of stomach of *M. posthuma*	Bacterial load of intestine of *M. posthuma*
Bacterial load of gizzard of *M. posthuma*	Pearson Correlation	1	.683(*)	.685(*)
	Sig. (2-tailed)	.	.014	.014
	N	12	12	12
Bacterial load of stomach of *M. posthuma*	Pearson Correlation	.683(*)	1	.678(*)
	Sig. (2-tailed)	.014	.	.015
	N	12	12	12
Bacterial load of intestine of *M. posthuma*	Pearson Correlation	.685(*)	.678(*)	1
	Sig. (2-tailed)	.014	.015	.
	N	12	12	12

* Correlation is significant at the 0.05 level (2-tailed).

Appendix 37: Correlations among bacterial load of gizzard, stomach and intestine of *E. incommodus*

		Bacterial load of gizzard of *E. incommodus*	Bacterial load of stomach of *E. incommodus*	Bacterial load of intestine of *E. incommodus*
Bacterial load of gizzard of *E. incommodus*	Pearson Correlation	1	.970(**)	.712(**)
	Sig. (2-tailed)	.	.000	.009
	N	12	12	12
Bacterial load of stomach of *E. incommodus*	Pearson Correlation	.970(**)	1	.800(**)
	Sig. (2-tailed)	.000	.	.002
	N	12	12	12
Bacterial load of intestine of *E. incommodus*	Pearson Correlation	.712(**)	.800(**)	1
	Sig. (2-tailed)	.009	.002	.
	N	12	12	12

** Correlation is significant at the 0.01 level (2-tailed).

Appendix 38: Correlations among bacterial load of gizzard, stomach and intestine of *E. nicholsoni*

		Bacterial load of gizzard of *E. nicholsoni*	Bacterial load of stomach of *E. nicholsoni*	Bacterial load of intestine of *E. nicholsoni*
Bacterial load of gizzard of *E. nicholsoni*	Pearson Correlation	1	.933(**)	.795(**)
	Sig. (2-tailed)	.	.000	.002
	N	12	12	12
Bacterial load of stomach of *E. nicholsoni*	Pearson Correlation	.933(**)	1	.919(**)
	Sig. (2-tailed)	.000	.	.000
	N	12	12	12
Bacterial load of intestine of *E. nicholsoni*	Pearson Correlation	.795(**)	.919(**)	1
	Sig. (2-tailed)	.002	.000	.
	N	12	12	12

** Correlation is significant at the 0.01 level (2-tailed).

Appendix 39: Correlation between total bacterial load of gut of six studied species of earthworm and factors of weather

		Total bacterial load of gut of six studied species of earthworm.
Rain fall (Avg.)cm	Pearson Correlation	.669(*)
	Sig. (2-tailed)	.017
	N	12
Relative humidity(Avg.)%	Pearson Correlation	.817(**)
	Sig. (2-tailed)	.001
	N	12
Minimum temperature(Avg.)°C	Pearson Correlation	.698(*)
	Sig. (2-tailed)	.012
	N	12
Maximum temperature(Avg.)°C	Pearson Correlation	.326
	Sig. (2-tailed)	.302
	N	12
Soil temperature(Avg.)°C	Pearson Correlation	.582(*)
	Sig. (2-tailed)	.047
	N	12
Sunshine hour(Avg.)	Pearson Correlation	-.636(*)
	Sig. (2-tailed)	.026
	N	12

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

Appendix 40: Correlation between bacterial load of gut of *L. mauritii* and factors of weather

		Bacterial load of gizzard of *L. mauritii*	Bacterial load of stomach of *L. mauritii*	Bacterial load of intestine of *L. mauritii*
Rain fall (Avg.)cm	Pearson Correlation	.621(*)	.516	.510
	Sig. (2-tailed)	.031	.086	.090
	N	12	12	12
Relative humidity(Avg.)%	Pearson Correlation	.669(*)	.720(**)	.718(**)
	Sig. (2-tailed)	.017	.008	.009
	N	12	12	12
Minimum temperature(Avg.)°C	Pearson Correlation	.558	.452	.514
	Sig. (2-tailed)	.059	.140	.087
	N	12	12	12
Maximum temperature(Avg.)°C	Pearson Correlation	.233	.099	.170
	Sig. (2-tailed)	.466	.759	.597
	N	12	12	12
Soil temperature(Avg.)°C	Pearson Correlation	.439	.332	.391
	Sig. (2-tailed)	.153	.292	.209
	N	12	12	12
Sunshine hour(Avg.)	Pearson Correlation	-.593(*)	-.483	-.558
	Sig. (2-tailed)	.042	.112	.059
	N	12	12	12

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Appendix 41: Correlation between bacterial load of gut of *E. orientalis* and factors of weather

		Bacterial load of gizzard of *E. orientalis*	Bacterial load of stomach of *E. orientalis*	Bacterial load of intestine of *E. orientalis*
Rain fall (Avg.)cm	Pearson Correlation	.619(*)	.657(*)	.612(*)
	Sig. (2-tailed)	.032	.020	.034
	N	12	12	12
Relative humidity(Avg.)%	Pearson Correlation	.832(**)	.600(*)	.723(**)
	Sig. (2-tailed)	.001	.039	.008
	N	12	12	12
Minimum temperature(Avg.)°C	Pearson Correlation	.672(*)	.908(**)	.805(**)
	Sig. (2-tailed)	.017	.000	.002
	N	12	12	12
Maximum temperature(Avg.)°C	Pearson Correlation	.299	.697(*)	.513
	Sig. (2-tailed)	.345	.012	.088
	N	12	12	12
Soil temperature(Avg.)°C	Pearson Correlation	.569	.874(**)	.738(**)
	Sig. (2-tailed)	.054	.000	.006
	N	12	12	12
Sunshine hour(Avg.)	Pearson Correlation	-.545	-.502	-.465
	Sig. (2-tailed)	.067	.097	.128
	N	12	12	12

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Appendix 42: Correlation between bacterial load of gut of *D. limella* and factors of weather

		Bacterial load of gizzard of *D. limella*	Bacterial load of stomach of *D. limella*	Bacterial load of intestine of *D. limella*
Rain fall (Avg.)cm	Pearson Correlation	.485	.483	.426
	Sig. (2-tailed)	.110	.112	.167
	N	12	12	12
Relative humidity(Avg.)%	Pearson Correlation	.759(**)	.766(**)	.709(**)
	Sig. (2-tailed)	.004	.004	.010
	N	12	12	12
Minimum temperature(Avg.)°C	Pearson Correlation	.479	.474	.400
	Sig. (2-tailed)	.115	.120	.198
	N	12	12	12
Maximum temperature(Avg.)°C	Pearson Correlation	.124	.118	.065
	Sig. (2-tailed)	.700	.715	.841
	N	12	12	12
Soil temperature(Avg.)°C	Pearson Correlation	.380	.375	.315
	Sig. (2-tailed)	.224	.229	.318
	N	12	12	12
Sunshine hour(Avg.)	Pearson Correlation	-.397	-.386	-.275
	Sig. (2-tailed)	.201	.216	.386
	N	12	12	12

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Appendix 43: Correlation between bacterial load of gut of *M. posthuma* and factors of weather

		Bacterial load of gizzard of *M. posthuma*	Bacterial load of stomach of *M. posthuma*	Bacterial load of intestine of *M. posthuma*
Rain fall (Avg.)cm	Pearson Correlation	.597(*)	.529	.744(**)
	Sig. (2-tailed)	.040	.077	.006
	N	12	12	12
Relative humidity(Avg.)%	Pearson Correlation	.658(*)	.814(**)	.593(*)
	Sig. (2-tailed)	.020	.001	.042
	N	12	12	12
Minimum temperature(Avg.)°C	Pearson Correlation	.502	.528	.929(**)
	Sig. (2-tailed)	.096	.077	.000
	N	12	12	12
Maximum temperature(Avg.)°C	Pearson Correlation	.188	.148	.715(**)
	Sig. (2-tailed)	.558	.647	.009
	N	12	12	12
Soil temperature(Avg.)°C	Pearson Correlation	.389	.419	.868(**)
	Sig. (2-tailed)	.211	.176	.000
	N	12	12	12
Sunshine hour(Avg.)	Pearson Correlation	-.568	-.487	-.655(*)
	Sig. (2-tailed)	.054	.108	.021
	N	12	12	12

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

Appendix 44: Correlation between bacterial load of gut of *E. incommodus* and factors of weather

		Bacterial load of gizzard of *E. incommodus*	Bacterial load of stomach of *E. incommodus*	Bacterial load of intestine of *E. incommodus*
Rain fall (Avg.)cm	Pearson Correlation	.672(*)	.732(**)	.637(*)
	Sig. (2-tailed)	.017	.007	.026
	N	12	12	12
Relative humidity(Avg.)%	Pearson Correlation	.854(**)	.882(**)	.743(**)
	Sig. (2-tailed)	.000	.000	.006
	N	12	12	12
Minimum temperature(Avg.)°C	Pearson Correlation	.549	.634(*)	.629(*)
	Sig. (2-tailed)	.065	.027	.028
	N	12	12	12
Maximum temperature(Avg.)°C	Pearson Correlation	.152	.225	.262
	Sig. (2-tailed)	.638	.482	.410
	N	12	12	12
Soil temperature(Avg.)°C	Pearson Correlation	.430	.499	.483
	Sig. (2-tailed)	.163	.099	.111
	N	12	12	12
Sunshine hour(Avg.)	Pearson Correlation	-.493	-.586(*)	-.799(**)
	Sig. (2-tailed)	.103	.045	.002
	N	12	12	12

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

Appendix 45: Correlation between bacterial load of gut of *E. nicholsoni* and factors of weather

		Bacterial load of gizzard of *E. nicholsoni*	Bacterial load of stomach of *E. nicholsoni*	Bacterial load of intestine of *E. nicholsoni*
Rain fall (Avg.)cm	Pearson Correlation	.566	.658(*)	.795(**)
	Sig. (2-tailed)	.055	.020	.002
	N	12	12	12
Relative humidity(Avg.)%	Pearson Correlation	.821(**)	.840(**)	.850(**)
	Sig. (2-tailed)	.001	.001	.000
	N	12	12	12
Minimum temperature(Avg.)°C	Pearson Correlation	.490	.678(*)	.765(**)
	Sig. (2-tailed)	.106	.015	.004
	N	12	12	12
Maximum temperature(Avg.)°C	Pearson Correlation	.107	.308	.376
	Sig. (2-tailed)	.741	.330	.228
	N	12	12	12
Soil temperature(Avg.)°C	Pearson Correlation	.378	.588(*)	.645(*)
	Sig. (2-tailed)	.225	.044	.023
	N	12	12	12
Sunshine hour(Avg.)	Pearson Correlation	-.415	-.537	-.771(**)
	Sig. (2-tailed)	.180	.072	.003
	N	12	12	12

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).